National Library of Canada
Bibliothèque nationale du Canada

Canadian Theses Service
Service des thèses canadiennes

Ottawa, Canada
K1A 0N4

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

NL-339 (r. 88/04) c

Canada

UNIVERSITY OF ALBERTA

THE EFFECT OF CECALLY-INFUSED PROPIONATE ON SERUM LIPIDS IN PIGS

BY

KATHLEEN ELIZABETH BEAULIEU

A THESIS SUBMITTED TO
THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR

THE DEGREE OF
MASTER OF SCIENCE
IN
NUTRITION

DEPARTMENT OF FOODS AND NUTRITION

EDMONTON, ALBERTA

FALL, 1990

National Library of Canada
Bibliothèque nationale du Canada

Canadian Theses Service
Service des thèses canadiennes

Ottawa, Canada
K1A 0N4

The author has granted an irrevocable non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of his/her thesis by any means and in any form or format, making this thesis available to interested persons.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without his/her permission.

L'auteur a accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

L'auteur conserve la propriété du droit d'auteur qui protège sa thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

ISBN 0-315-65080-X

UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR: KATHLEEN ELIZABETH BEAULIEU

TITLE OF THESIS: THE EFFECT OF CECALLY-INFUSED PROPIONATE ON SERUM LIPIDS IN PIGS

DEGREE: MASTER OF SCIENCE IN NUTRITION

YEAR THIS DEGREE GRANTED: 1990

PERMISSION IS HEREBY GRANTED TO THE UNIBERSITY OF ALBERTA LIBRARY TO REPRODUCE SINGLE COPIES OF THIS THESIS AND TO LEND OR SELL SUCH COPIES FOR PRIVATE, SCHOLARLY OR SCIENTIFIC RESEARCH PURPOSES ONLY.

THE AUTHOR RESERVES OTHER PUBLICATION RIGHTS, AND NEITHER THE THESIS NOR EXTENSIVE EXTRACTS FROM IT MAY BE PRINTED OR OTHERWISE REPRODUCED WITHOUT THE AUTHOR'S WRITTEN PERMISSION.

KATHLEEN ELIZABETH BEAULIEU

1941 ROYAL CRESCENT

LONDON, ONTARIO

CANADA

N5V 1N6

DATE: _____

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

THE UNDERSIGNED CERTIFY THEY HAVE READ, AND RECOMMEND TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH FOR ACCEPTANCE, A THESIS ENTITLED:

THE EFFECT OF CECALLY-INFUSED PROPIONATE ON SERUM LIPIDS IN PIGS

SUBMITTED BY KATHLEEN ELIZABETH BEAULIEU IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN NUTRITION.

Michael I. McBurney

DR. MICHAEL I. McBURNEY, SUPERVISOR

DR. M. TOM CLANDININ

DR. ZENIA HAWRYSH

DR. WILLEM C. SAUER

DATE: October 11, 1990

FOR MICHAEL EMERSON BEAULIEU

TO WHOM I WILL DEDICATE SO MUCH MORE THAN THIS

ABSTRACT

Studies have shown the dietary supplementation of water-soluble fiber to be more effective at serum cholesterol lowering than the supplementation of water-insoluble fiber. Water-soluble fiber sources are more easily fermented by colonic bacteria than water-insoluble fiber sources. Short chain fatty acids are major end products of colonic fiber fermentation. It has been hypothesized that colonic absorption of the 3-carbon short chain fatty acid (propionate) is responsible for the observed hypocholesterolemic effect of water-soluble fiber supplementation.

To test this hypothesis, 9 barrows (35 kg) were surgically fitted with a T-cannula at the distal ileum and a cecal cannula. Pigs were fed a high saturated fat diet (10% fat w/w, 109 g feed/$kg^{0.75}$ per day). Pigs were continually infused for 14 day periods with either propionate (36 mmol/$kg^{0.75}$ per day) or physiological saline at a rate of 1.05 ml/min in a crossover design. Blood samples were collected by jugular puncture after each infusion period. Serum lipoproteins were separated by density using sequential floatation ultracentrifugation. Serum triglyceride, total cholesterol, VLDL cholesterol, LDL cholesterol, and HDL cholesterol were determined enzymatically. Fecal samples (72 hour) and ileal digesta samples (24 hour) were collected during both periods to determine nutrient digestibility and

bile acid content. Serum triglyceride levels (mean ± SEM) were 50.5 ± 3.0 mg/dl and 43.3 ± 4.2 mg/dl after propionate and saline infusions respectively ($p \leq 0.17$). Serum cholesterol was higher after propionate infusion (133.9 ± 4.3 mg/dl) than after saline infusion (116.7 ± 4.2 mg/dl) ($p \leq 0.011$). LDL cholesterol was higher after propionate infusion (72.8 ± 3.1 mg/dl) than after saline infusion (63.2 ± 1.8 mg/dl) ($p \leq 0.017$). Differences in VLDL cholesterol and HDL cholesterol were not detected between infusion treatments.

Differences in nutrient digestibilities were not detected between treatments. The pooled digestibilities were 79.0% ± 2.0% and 84.0% ± 0.5% for ileal and fecal nitrogen, and 75.1% ± 1.8% and 87.1% ± 0.03% for ileal and fecal organic matter, respectively. Differences in ileal and fecal bile acid, cholesterol, and total sterol contents were not detected between treatments. VLDL cholesterol was negatively correlated with ileal cholesterol ($p \leq 0.04$), and apparent colonic disappearance of cholesterol ($p \leq 0.03$). Differences in liver cholesterol were not detected between treatments.

The increase in serum cholesterol after propionate infusion, primarily in LDL cholesterol, was not attributable to changes in nutrient digestibility, or sterol excretion. It cannot be concluded that cecally-derived propionate is responsible for the serum lipid lowering effect observed in water-soluble fiber supplementation studies.

ACKNOWLEDGEMENTS

I would like to thank Dr. M.T. Clandinin, Dr. Z. Hawrysh, and Dr. W.C. Sauer for serving as members of my committee. Special thanks are extended to my supervisor, Dr. Mike McBurney, a true scientist whose enthusiasm and knowledge provide an atmosphere conducive to new ideas.

Gratitude is expressed to Dr. Sauer and Mike Dugan for performing the cannulation surgeries. The artistic assistance from Linda Turner is gratefully acknowledged. Thanks are expressed to Dr. Goh for sharing his expertise in serum lipid analysis. Thanks are extended to Leigh-Ann Carrothers for the patience and kindness she extended to the animals while she assisted with the experiment. The participation of Arnie, Jake, Wilbur, Alf, Xavier, Caption, Mr.T, Schizo, and Kennedy is respectfully acknowledged. Gratitude is extended to NSERC for the financial support that made this research possible.

To my parents and family in London: words cannot express my thanks for the strength you give me to pursue my dreams.

TABLE OF CONTENTS

		PAGE
INTRODUCTION		1
LITERATURE REVIEW		3
1	TYPES OF FIBER	3
2	UPPER INTESTINAL EFFECTS	5
3	COLONIC EFFECTS	6
4	EFFECT OF FIBER ON SERUM LIPIDS	9
5	THE EFFECT OF BILE ACID BINDING ON SERUM LIPIDS	14
6	SHORT CHAIN FATTY ACIDS	16
	6.1 PRODUCTION	16
	6.2 TRANSPORT ACROSS THE GUT	18
	6.3 METABOLISM	20
	6.4 THE EFFECT OF PROPIONATE ON SERUM LIPIDS	24
7	CHOICES OF AN ANIMAL MODEL	26
	7.1 PHYSIOLOGICAL SIMILARITIES BETWEEN HUMAN AND PORCINE GUT FUNCTION	30
	7.2 SIMILARITIES BETWEEN HUMAN AND PORCINE LIPOPROTEINS	35
	7.3 THE CANNULATED PIG MODEL	37
HYPOTHESIS		38
METHODOLOGY		39
1	ANIMALS	39

2 CANNULATION 39
2.1 SURGERY 39
2.2 RECOVERY 43
3 EXPERIMENTAL DESIGN 43
3.1 INFUSION PERIODS 44
3.1.1 SALINE INFUSION 46
3.1.2 PROPIONATE INFUSION 46
3.2 COLLECTIONS 47
3.2.1 ILEAL DIGESTA COLLECTION 47
3.2.2 FECAL COLLECTION 48
3.2.3 BLOOD COLLECTION 49
3.2.4 LIVER COLLECTION 49
4 DIETS 49
4.1 CONSUMPTION 49
4.2 COMPOSITION 50
4.3 LABORATORY ANALYSIS OF DIET 53
4.3.1 MOISTURE 53
4.3.2 ASH 54
4.3.3 CHROMIC OXIDE 54
4.3.4 NITROGEN 56
4.3.5 ORGANIC MATTER 58
4.3.6 CHOLESTEROL 58
4.3.6.1 EXTRACTION AND METHYLATION 59
4.3.6.2 ENZYMATIC ASSAY FOR CHOLESTEROL DETERMINATION ... 59

5 ILEAL DIGESTA AND FECAL ANALYSIS 61
5.1 MOISTURE 61
5.2 ASH 61
5.3 CHROMIC OXIDE 61
5.4 NITROGEN 62
5.5 ORGANIC MATTER 63
5.6 CHOLESTEROL 63
5.7 BILE ACID 65
5.7.1 EXTRACTION 65
5.7.2 REMOVAL OF LIPIDS FROM EXTRACT 66
5.7.3 ENZYMATIC ASSAY FOR BILE ACIDS 67
6 SERUM ANALYSIS 68
6.1 TRIGLYCERIDE 68
6.2 TOTAL CHOLESTEROL 70
6.3 LIPOPROTEIN CHOLESTEROL 71
6.3.1 VLDL 72
6.3.1.1 DENSITY ADJUSTMENT 72
6.3.1.2 CENTRIFUGATION AND ISOLATION 73
6.3.1.3 CHOLESTEROL ANALYSIS 74
6.3.2 LDL 74
6.3.2.1 DENSITY ADJUSTMENT 74
6.3.2.2 CENTRIFUGATION AND ISOLATION 75
6.3.2.3 CHOLESTEROL ANALYSIS 76

6.3.3 HDL 76

6.3.3.1 DENSITY ADJUSTMENT 76

6.3.3.2 CENTRIFUGATION AND ISOLATION 77

6.3.3.3 CHOLESTEROL ANALYSIS 78

7 LIVER ANALYSIS 78

7.1 TOTAL CHOLESTEROL CONTENT 78

8 STATISTICAL ANALYSIS 79

RESULTS 82

DISCUSSION 101

REFERENCES 113

LIST OF TABLES

PAGE

TABLE 1 Classification of dietary fiber and fiber sources 4

TABLE 2 Gut bacterial composition in humans and pigs 8

TABLE 3 Serum lipid response to water-soluble fiber supplementation 11

TABLE 4 Relative volume of gastrointestinal segments in the pig and human 32

TABLE 5 Ratios of short chain fatty acids found in the feces of humans and pigs 33

TABLE 6 Formulation of standard grower diet and high fat diet 51

TABLE 7 Nutrient content of selected nutrients in the grower diet and the high fat diet 52

TABLE 8 Weight gain, weight change, and feed efficiency 84

TABLE 9 Ileal and fecal digestibility coefficients of nitrogen and organic matter 85

TABLE 10 Serum cholesterol content, triglyceride content and LDL/HDL cholesterol ratio 86

TABLE 11 Liver cholesterol and liver weight 89

TABLE 12 Sterol content of ileal digesta and feces 91

TABLE 13 Apparent colonic disappearance of sterols 92

TABLE 14 Pearson correlation coefficient matrix for serum lipids 93

TABLE 15 Pearson correlation coefficient matrix for serum lipids, ileal and fecal sterol content, and apparent colonic disappearance 94

TABLE 16 Pearson correlation coefficient matrix for fecal and ileal sterol content 96

TABLE 17 Pearson correlation coefficient matrix for ileal, fecal, and apparent colonic disappearance of sterols 97

TABLE 18 Pearson correlation coefficient matrix for liver weight, liver cholesterol and serum lipids 98

TABLE 19 Pearson correlation coefficient matrix for liver weight and cholesterol with ileal content, fecal content, and apparent colonic disappearance of sterols 100

LIST OF FIGURES

PAGE

FIGURE 1 Proposed mechanism of short chain fatty acid transport into mucosal cells 19

FIGURE 2 Formation of ketone bodies from acetate and butyrate 22

FIGURE 3 Formation of succinate from propionate 23

FIGURE 4 Utilization of 3-hydroxy-3-methylglutaryl-CoA in formation of mevalonic acid for cholesterol synthesis 27

FIGURE 5 The distal ileal T-cannula 41

FIGURE 6 The cecal infusion cannula 42

FIGURE 7 Study design 45

FIGURE 8 Pig weights 83

FIGURE 9 Effect of infusion treatment on serum lipids 88

LIST OF ABBREVIATIONS

°C = degrees celsius

GI = gastrointestinal

h = hour

HDL = high density lipoprotein

HMG-CoA = (3)-hydroxy-(3)-methylglutaryl coenzyme A

Ho = hypothesis

LDL = low density lipoprotein

min = minute

n = number of experimental units

p = probability

R = Pearson correlation coefficient

SCFA = short chain fatty acid

SEM = standard error of the mean

VLDL = very low density lipoprotein

INTRODUCTION

The increased consumption of dietary fiber has been significantly correlated to a decreased incidence of coronary heart disease, obesity, and atherosclerosis (Burkitt and Trowell, 1975). Research through controlled studies has confirmed many beneficial health effects result from high fiber diets, although mechanisms of action remain unclear.

The American Diabetic Association (1988), and American Heart Association (1984) have recommended generous fiber intakes of 20 - 30 g/day for those afflicted with diabetes and cardiovascular disease based on the accumulation of evidence in the last 15 years. The consumption of fiber for prevention of disease states is also considered important (Health and Welfare Canada, 1985; Food and Drug Administration, 1987).

Studies of high fiber diets have shown that water-soluble fibers exhibit more consistent effects on lowering serum lipid levels than water-insoluble fibers (Anderson and Chen, 1979; Kies, 1985). Water-soluble fibers are usually more easily fermented in the colon (McBurney and Thompson, 1989). The major non-gaseous organic end products of fiber fermentation in the colon are acetate, propionate, and butyrate. These short chain fatty acids (SCFAs) are absorbed

by mucosal cells and can be utilized as energy by the gut or metabolized by the liver. Enhanced SCFA production and absorption may be responsible for some of the beneficial health effects of dietary fiber.

The odd-number carbon chain structure of propionate may alter cholesterol synthesis through decreased hepatic HMG-CoA synthase activity (Bush and Milligan, 1971; Thacker et al, 1981; Lowe and Tubbs, 1985). This enzyme mediates formation of a precursor to cholesterol synthesis. Another proposed mechanism for the observed serum lipid lowering effect of dietary fiber is that bile acids and other sterols bind to fiber in the small intestine, preventing reabsorption (Kay and Truswell, 1977; Vahouny et al, 1980). Enhanced bile acid excretion would decrease the sterol pool in the body, and may decrease serum cholesterol.

The effect of colonic propionate absorption on serum lipids cannot be separated from the upper GI effects of fiber consumption when high fiber diets are studied. An alternate model must be used to examine the effect of propionate on serum cholesterol to determine the contribution of propionate to the serum lipid lowering effects of fermentable fiber consumption.

LITERATURE REVIEW

1 . TYPES OF FIBER

Mechanisms of action for the observed benefits of increased fiber consumption will be more easily understood if characteristics of different types of fibers are distinguished and individually assessed. Recommended intakes do not presently differentiate between types of fiber because of conflicting reports as to whether some fiber sources are more beneficial than others in promoting certain health effects. There is also uncertainty in analytical determination and categorization of the type of fiber a food contains (Bingham, 1987).

Dietary fiber is defined as plant polysaccharides, lignin, and other plant components such as waxes and cutins that are resistant to hydrolysis by the digestive enzymes of man (Trowell et al, 1976). Non-starch polysaccharides (NSP) can be divided into water-soluble and water-insoluble fractions. Lignin is a water-insoluble fiber. Table 1 shows a categorization of these fibers and their main sources (Anderson, 1986). The solubility of these fibers distinguishes them analytically but does not necessarily group them according to physiological effects.

TABLE 1

Classification of dietary fiber and fiber sources[1]

Type of fiber	Plant component	Major food sources
Insoluble		
Non-carbohydrates	Lignin	-all plants -mainly whole grains, seeds, and woody parts of vegetables
Carbohydrate	Cellulose Hemi-cellulose	-vegetables, fruits -wheat, bran, rye, and whole grains
Soluble		
Carbohydrate	Pectins Gums	-citrus fruits and vegetables -oat products and legumes

[1] Adapted from Anderson, 1986.

2 UPPER INTESTINAL EFFECTS

Trace minerals such as Fe, Zn, Cu, and Ca have been shown to bind to certain dietary fibers in the small intestine, decreasing their availability to the absorptive surfaces of the intestinal mucosa (Platt and Clydesdale, 1985; Garcia-Lopez and Lee, 1985). Functional groups of the fiber molecules can bind metals at specific or non-specific binding sites (Platt and Clydesdale, 1987).

Malabsorption of starch in the upper GI has also been shown to result from fiber supplementation, providing starch as a fermentable substrate to the microbes in the colon. A more viscous and bulky matrix is formed in the small intestine upon fiber consumption, holding some of the nutrient content away from the absorptive surfaces of the mucosa. Although a delay in gastric emptying has been attributed to the increased viscosity of digesta when water-soluble fibers are consumed (Leeds et al, 1981), this does not result in enhanced absorption of nutrients.

Sterols such as cholesterol and bile acids may bind to fibers in the small intestine, resulting in decreased absorption or reabsorption of these molecules (Bosaeus et al, 1986; Hillman et al, 1986; Bosaeus and Andersson, 1987). In studies with human ileostomy patients, the addition of pectin

to low fiber diets increased bile acid and cholesterol content of the ileal effluent, indicative of reduced upper intestinal absorption of bile acids and dietary cholesterol (Bosaeus et al, 1986).

3 COLONIC EFFECTS

Water-insoluble fibers such as wheat bran have been found to increase fecal bulk (Cummings, 1982). Most of this increase is attributable to the unfermented cellulosic fiber content of the feces (Cummings et al, 1978). The resulting large, wet stools and decreased intestinal transit time (Roehrig, 1988) are laxative properties mainly observed upon water-insoluble fiber ingestion (Kochen et al, 1985), although water-soluble types have also been shown to be effective as laxatives (Smith et al, 1980). Decreased intestinal transit time is also associated with water-soluble fiber supplementation (Roehrig, 1988) but a greater decrease is seen with water-insoluble fibers.

Fermentable fibers such as pectins and gums increase fecal nitrogen content due to the increased presence of bacterial cells (Cummings et al, 1979). As very little undigested fiber is present in feces, an increase in stool size is not always seen (Nyman and Asp, 1982; Penagini et al, 1986). Increased fecal fat, sterol, and bile acid content can

also occur with supplementation of a water-soluble fiber source (Reddy et al, 1980; Kelley et al, 1981).

Upon passage of dietary fiber into the colon, gut bacteria partially or wholly degrade the fiber. The bacteria of the human gut are mostly anaerobic, saccharolytic organisms (Table 2) and comprise 55% of total fecal solids (Stephen and Cummings, 1980). Many substrates passed on from the small intestine can be degraded by bacteria in the colon. Fiber fractions, malabsorbed starches or proteins, and mucins can be used as substrates for colonic organisms (Grimble, 1989). High fiber diets that provide fermentable substrate to the colon stimulate colonic microbial proliferation.

The primary fermentation products of fiber are carbon dioxide, methane, hydrogen, water, and short chain fatty acids (SCFAs) (Miller and Wolin, 1979). The majority of SCFAs produced are acetate, propionate, and butyrate which are organic acids containing 2, 3, and 4 carbons, respectively (Hoverstad, 1986). All dietary fibers are not equal as fermentable substrates. Generally, water-soluble fibers are more fermentable than water-insoluble fibers. Quantities of total SCFAs produced per gram of dietary fiber as well as the ratio of these acids (acetate : propionate : butyrate) differ among fiber sources when fermented in vitro with human fecal

TABLE 2

Gut bacterial composition in humans and pigs

Human (% population)[1]		Pig (% population)[2]	
Bacteroides fragilis ss vulgaris	11.8	Bacteroides ruminicola	33.5
Eubacterium aerofaciens	9.9	Selenomonas ruminantium	20.8
Bacteroides fragilis ss thetaiotaomicron	8.9	Lactobacillus acidophilus	7.6
Peptostreptococcus productus	9.4	Butyrivibrio fibrisolvens	6.1
Bacteroides fragilis ss distasonis	6.0	Peptostreptococcus productus	3.0
Fusobacterium prausitzii	4.4	Bacteroides uniformis	3.0
Coprococcus eutactus	3.5	Eubacterium aerofaciens	2.5
Ruminococcus bromii	2.7	Lactobacillus fermentum	2.5
		Bacteroides multiacidus	2.0
		Fusobacterium prausitzii	2.0
		Megasphaera elsdenii	2.0

[1] Holdeman et al, 1976 - Average % of flora in 3 North American men

[2] Robinson et al, 1981 - Average % of flora in 3 pigs

microbiota (McBurney and Thompson, 1989; Tomlin et al, 1989). SCFA production and utilization is discussed further in Section 4.

4 EFFECT OF FIBER ON SERUM LIPIDS

Much emphasis in fiber research has been placed on hypotriglyceridemic and hypocholesterolemic effects. Results, though inconsistent, have implications for treatment of hyperlipidemic related conditions.

Feeding 15 g citrus pectin per day for 21 days decreased the serum cholesterol of nine normolipidemic human subjects by 13% ($p \leq 0.001$) (Kay and Truswell, 1977). The addition of 5% pectin to a high cholesterol diet for 28 days reduced serum cholesterol in rats by 9% ($p \leq 0.05$) and liver cholesterol by 27% ($p \leq 0.05$) (Leveille and Sauberlich, 1966). Supplementing grapefruit pectin to miniature swine on high cholesterol diets reduced plasma cholesterol by 32% ($p \leq 0.05$) (Baekey et al, 1987). Other studies have found a serum lipid lowering effect of pectin-containing diets (Keys et al, 1961; Wells and Ershoff, 1962; Palmer and Dixon, 1966; Kiriyama et al, 1969; Durrington et al, 1976; Langley and Thye, 1977; Miettinen and Tarpila, 1977; Jenkins et al, 1979).

Twelve hyperlipidemic humans fed 30 g guar gum per day in a double blind placebo controlled crossover experiment showed a 9.6% decrease in serum cholesterol ($p \leq 0.05$) (Turner et al, 1990). In six healthy subjects, serum cholesterol decreased by 16% ($p \leq 0.05$) after a two week supplementation period providing 5.7 g guar per day (Penagini et al, 1986). These results are in agreement with serum lipid lowering effects of guar found in other studies (Jenkins et al, 1979; Jenkins et al, 1980; Khan et al, 1981; Simons et al, 1982; McLean Ross et al, 1983; Tuomilehto et al, 1983; Tuomilehto et al, 1988).

Some results of experiments studying the effect of oat and bean product consumption on humans serum cholesterol are shown in Table 3 (Anderson and Gustafson, 1988). Oat bran supplementation (87 g per day) in normolipidemic humans did not significantly lower serum cholesterol compared to isocaloric wheat starch supplementation for a six week experimental period (Swain et al, 1990). However, after both treatments subjects had about 10% lower serum cholesterol than before supplementation ($p \leq 0.05$). Oat bran supplementation (100 g/day) in 7 hyperlipidemic men for 10 days showed a 13% decrease in serum cholesterol, mainly in the LDL fraction (Kirby et al, 1981)

TABLE 3

Serum lipid response to water-soluble fiber supplementation

Fiber source	Quantity (g/day dry wt)	Duration (weeks)	Subject lipid status[1]	Serum lipids[3] (%change from control)			
				CH	LDL	HDL	TG
oat bran[a]	100	1	HL	-13	-14	(NS)	-5
oat bran[b]	100	3	HL	-19	-23	(NS)	-19
oat bran[c]	100	3	HL	-23	-23	-20	-21
oat bran[d]	50	6	N	-12	--	--	(NS)
canned beans[e]	140[2]	16	HL	-7	(NS)	(NS)	-25
dried beans[b]	115	3	HL	-19	-24	(NS)	(NS)
dried beans[c]	100	3	HL	-23	-23	(NS)	-21
canned beans[f]	122[2]	3	HL	-13	--	--	(NS)
oat bran +beans[b]	41 145[2]	24	HL	-26	-24	(NS)	-25
oat bran +beans[b]	41 145[2]	99	HL	-22	-29	(NS)	--
guar gum[g]	6	2	N	-16	--	--	(NS)

[1] HL - hyperlipidemic, N - normal
[2] wet weight
[3] CH - cholesterol, TG - triglyceride
-- - results not recorded, NS - non-significant results

[a]-Kirby et al, 1981
[b]-Anderson et al, 1984(a)
[c]-Anderson et al, 1984(b)
[d]-Storch et al, 1984
[e]-Jenkins et al, 1983
[f]-Anderson, 1985
[g]-Penagini et al, 1986

Most of the interest lies in lowering blood lipids for hyperlipidemic subjects. Studies examining lipid alterations in normal lipid status subjects have found less consistent results. Lipid control mechanisms may be more resistant to change in healthy subjects whose minimum serum cholesterol may already be achieved, than in subjects with impaired lipid control (Kies, 1985).

The results of wheat bran or cellulose supplementation studies have shown a general lack of serum lipid lowering effects (Kiriyama et al, 1969; Balmer and Zilversmit, 1974; Anderson and Chen, 1979). A comparison of wheat bran and pectin supplementation in humans showed no effect of wheat bran, but a 5% ($p \leq 0.05$) decrease in serum cholesterol after pectin supplementation (Keys et al, 1961). No cholesterol lowering effect was found in rats consuming high cholesterol diets supplemented with 30% wheat bran compared to the low fiber diet control rats (Arvanitakis et al, 1977).

Water-soluble fibers such as pectins, guar gum, oat products and bean products illustrate a more consistent effect on lowering serum lipids than water-insoluble fibers such as cellulose and wheat bran (Tsai et al, 1976; Anderson and Chen, 1979; Story, 1985; Vigne et al, 1987).

Water soluble fibers are more completely fermented upon in vitro fermentation by colonic bacteria (McBurney and Thompson, 1989). Inhibition of cholesterol synthesis and enhanced gluconeogenesis are hypothesized results of SCFA uptake from colonic fiber fermentation. This is in agreement with the apparent enhanced hypocholesterolemic effect of the more fermentable fibers.

Lowered cholesterol levels may be due to enhanced bile acid and sterol excretion. Bile acids may bind to fibers in the small intestine resulting in impaired reabsorption (Bosaeus et al, 1986; Hillman et al, 1986; Bosaeus and Andersson, 1987). Synthesis of replacement bile acids from the sterol pool in the body would lower serum cholesterol levels.

HDL cholesterol is generally not as consistently altered by fiber supplementation as is LDL cholesterol. Changes in total serum cholesterol are the most consistent and pronounced. Low density lipoproteins, composed of 45% cholesterol, contain more cholesterol than any other transport lipoprotein (Whitney and Hamilton, 1987). Lowered LDL cholesterol would be expected if total serum cholesterol were markedly decreased. As LDL is mainly responsible for cholesterol deposition to tissues, a decrease in serum quantities infers lowered risk of atherosclerosis.

5 THE EFFECT OF BILE ACID BINDING ON SERUM LIPIDS

It has been shown that dietary fibers adsorb bile acids and may also bind dietary cholesterol in the small intestine, reducing reabsorption or absorption of these sterols (Vahouny et al, 1980). The resulting enhanced sterol excretion may reduce body sterol pools as more hepatic sterols are converted to bile acids to replace losses. This may result in a lowering of serum lipids. However, bile acid excretion does not consistently correlate with decreased serum cholesterol.

The correlation of bile acid binding capacity with serum lipid lowering effects of fiber has not been clearly established. In vitro binding capacities for bile acid binding showed lignin, guar gum, and pectin to have high affinity, and cellulose and wheat bran to have low affinity (Vahouny et al, 1980; Hillman et al, 1986). Variations in methods and in vitro conditions provide discrepancies between the results of in vitro binding capacity studies.

The effect of fiber supplemented meals on small intestinal bile acid contents was studied in rats fed single meals of either cellulose, wheat bran, oat bran, guar gum, lignin, cholestryamine (a bile acid sequestrant), or no supplement (Gallaher and Schneeman, 1986). Total upper-intestinal bile acid contents of the treatment groups two

hours post-meal were not significantly different from the control group with the exception of the lignin diet that showed a 60% increase in total bile acid content. Effects on blood lipids were not determined.

Rats consuming diets supplemented with either pectin, guar gum, psyllium, cellulose, wheat bran, alfalfa, cholestryamine or no supplement for a four week period showed that bile acid output was significantly higher than the control in all supplemented diets except pectin (Vahouny et al, 1987). Serum lipids were not assessed.

Fecal sterol excretion over three days of wheat bran or pectin supplementation in the diets of ileostomy patients was studied by Bosaeus et al, 1986. Results showed that fat, bile acid, and cholesterol excretion was increased only during pectin supplementation.

Hypercholesterolemic subjects supplemented with 15 g/day pectin showed decreases in plasma cholesterol and increases in bile acid, neutral sterol, and fat excretion at the end of a two week supplementation period (Kay and Truswell, 1977). This hypocholesterolemic response is consistent with many other fiber supplementation studies (Kirby et al, 1981; Anderson et al, 1984 (a)). It remains to be determined

whether enhanced sterol excretion occurs independent of hypocholesterolemic effects.

6 SHORT CHAIN FATTY ACIDS

The mechanisms by which dietary fiber supplementation results in beneficial health effects have yet to be elucidated. SCFAs may be important in the regulation of lipid metabolism. Upon review of recent studies, however, there is a lack of conclusive evidence to attribute health effects to SCFA production in the colon. A mechanistic approach is required to look at the effect of colonically-derived SCFAs distinct from upper intestinal effects of fiber consumption.

6.1 PRODUCTION

The quantities of SCFAs produced in the colon as bacterial fermentation products of polysaccharides vary proportionally with dietary fiber intake and the overall digestibility of the meal consumed. The stoichiometrically balanced equation for the colonic production of SCFAs, methane, water, and CO_2 from a 100% fermentable carbohydrate source was calculated (equation 1) (Miller and Wolin, 1979). Equation 1 assumes the production of methane although only an estimated one third of all humans have colonic methane producing bacteria (Bond et al, 1971). SCFAs are assumed to

be the sole non-gaseous organic end products of this reaction. The molar ratio of CO_2:(CH_4 + H_2O) is assumed to be 1:1 for the calculation of this equation (Brieger, 1878; Miller and Wolin, 1979).

$$34.5\ C_6H_{12}O_6 \text{ -----> } 64\ SCFA + 23.75\ CH_4 + 34.25\ CO_2 + 10.5\ H_2O \quad (1)$$

Equation 1 must be adjusted to the molar ratios of SCFAs found in vivo. The three major acids acetate, propionate, and butyrate are produced in an approximate molar ratio of 3.75 : 1.5 : 1 (Cummings, 1981). Equation 2 shows the equation for the physiological molar quantities of SCFAs produced in vivo.

$$335\ C_6H_{12}O_6 \text{ ---> } 360\ acetate + 144\ propionate + 96\ butyrate + 153\ CH_4 + 321\ CO_2 + 168\ H_2O \quad (2)$$

Conversion of equation 2 into weight quantities for estimation of SCFA production from a weight quantity of carbohydrate is shown in equation 3. The assumption of a constant molar ratio of acetate: propionate: butyrate is supported by in vitro fermentation studies of different fiber sources (McBurney and Thompson, 1989; Tomlin et al, 1989). It has been shown that more variation exists between total

quantities of SCFAs derived from fermentation of different fiber sources than in the ratios of the SCFAs produced (McBurney and Thompson, 1989).

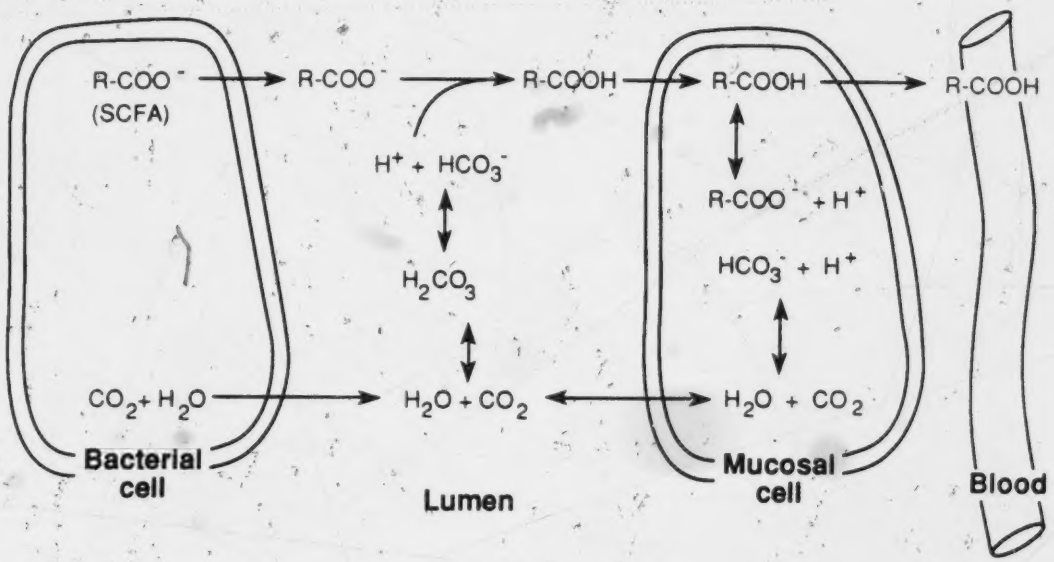
$$100g\ C_6H_{12}O_6 \text{ ------->} \begin{array}{l} 35.8g \text{ acetate (597 mmol)} \\ + 17.7g \text{ propionate (239 mmol)} \\ + 14.0g \text{ butyrate (159 mmol)} \\ + \ 4.1g\ CH_4 + 23.4g\ CO_2 \\ + 5.0g\ H_2O \end{array} \quad (3)$$

6.2 TRANSPORT ACROSS THE GUT

Short chain fatty acids produced in the colon are absorbed by the gut mucosal cells. The disappearance of SCFAs from the colon is coupled with the appearance of bicarbonate ions (HCO_3^-) in the lumen (Ruppin et al, 1980). This absorption process has not been fully characterized, but it has been hypothesized that more than 60% of the ionic form of the SCFA is protonated before entering the mucosal cells (Ruppin et al, 1980) in a process described in Figure 1. This mechanism accounts for the HCO_3^- accumulation observed in other work (Umesaki et al, 1979; Argenzio et al, 1977) as well as a subsequent increase in luminal pH upon SCFA absorption. This protonated form of the acid will more easily diffuse through the lipid membrane of the mucosal cell than will the ionic form of the acid (Hogben et al, 1959; Bougat, 1987).

FIGURE 1

Proposed mechanism of short chain fatty acid transport into mucosal cells[1]



[1] Adapted from Bougat, 1987

The energy provided from acetate, propionate, and butyrate is 209, 365, and 522 kcal/mol, respectively (CRC, 1984). It has been estimated that 540 kcal/day can be obtained through SCFA absorption in humans (Ruppin et al, 1980). Only 5% of SCFAs produced in the colon remain unabsorbed in the feces (McNeil et al, 1978).

6.3 METABOLISM

The utilization of these SCFAs after absorption is still unclear. Some acids absorbed from the gut are metabolized at the site of absorption in the mucosal cell (Hoverstad, 1986). Most of the remaining SCFAs are metabolized by the liver. Propionate and butyrate concentrations were found to be ten times greater while acetate was found to be four times greater in the portal blood than in the peripheral veinous blood indicating hepatic clearance (Dankert et al 1981).

Acetate is easily incorporated into ketone bodies and primarily metabolized to glutamate, acetoacetate and aspartate as shown by ^{14}C labelled acetate incorporation (Marty et al, 1985). These products can be easily utilized as energy. Butyrate is partially converted to acetate and ketone bodies in the lumen (Bougat, 1987) through oxidative cleavage of a 2 carbon unit from the four carbon chain.

Rombeau (1988) demonstrated an increase in whole segment colonic weight and colonic mucosal DNA in rats upon colonic infusion of mixed SCFAs relative to saline infusion. In vitro studies have shown that butyrate is taken up faster by mucosal cells than other substrates: acetoacetate, glutamine, and glucose (Roediger, 1982). SCFAs have also been shown to stimulate mucosal cell proliferation (Sakata, 1987). Both intravenous and intracecal infusion of SCFAs showed stimulation of mitotic activity in the jejunal and ileal mucosa in subjects on a total parenteral nutrition feeding regime (Koruda et al, 1987). A likely explanation for this stimulation of mucosal cell growth is the production of ketone bodies from SCFAs, and the availability of these substrates to the mucosal cells as oxidative fuels. An estimated 90% of the butyrate produced in the gut is utilized by the gut, leaving only 10% to be transported to the liver (Bergman, 1975). Figure 2 shows how even numbered carbon chain substrates such as acetate and butyrate can form acetoacetate or D-3-hydroxybutyrate. These end products (Figure 2 (9) and (10)) produce acetyl-CoA which is oxidized in the tricarboxylic acid (TCA) cycle to provide energy.

Propionate is metabolized to succinate which can enter the TCA cycle or go on to gluconeogenesis. The reactions outlined in Figure 3 show intermediates in the formation of

FIGURE 2

Formation of ketone bodies from acetate and butyrate

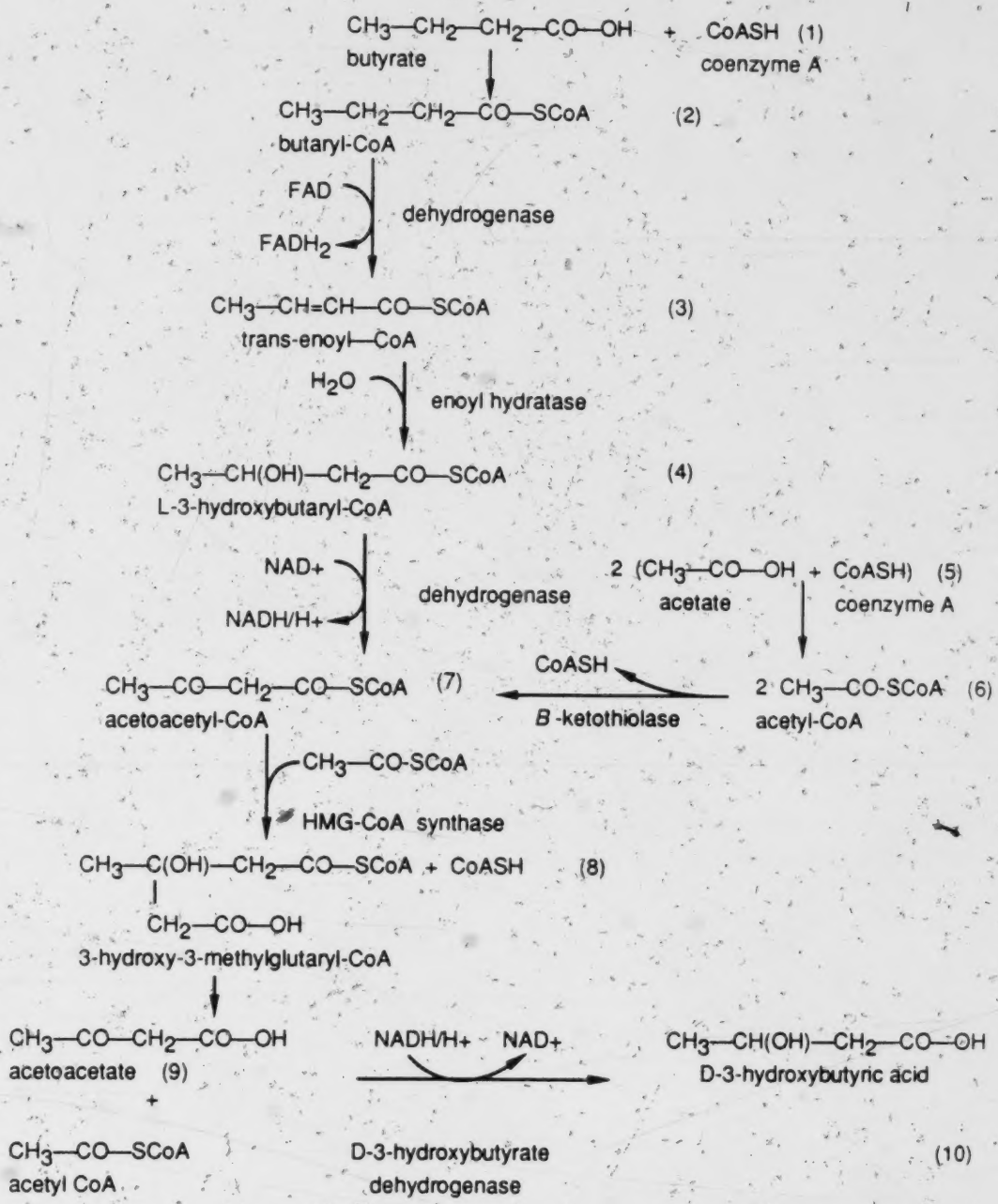
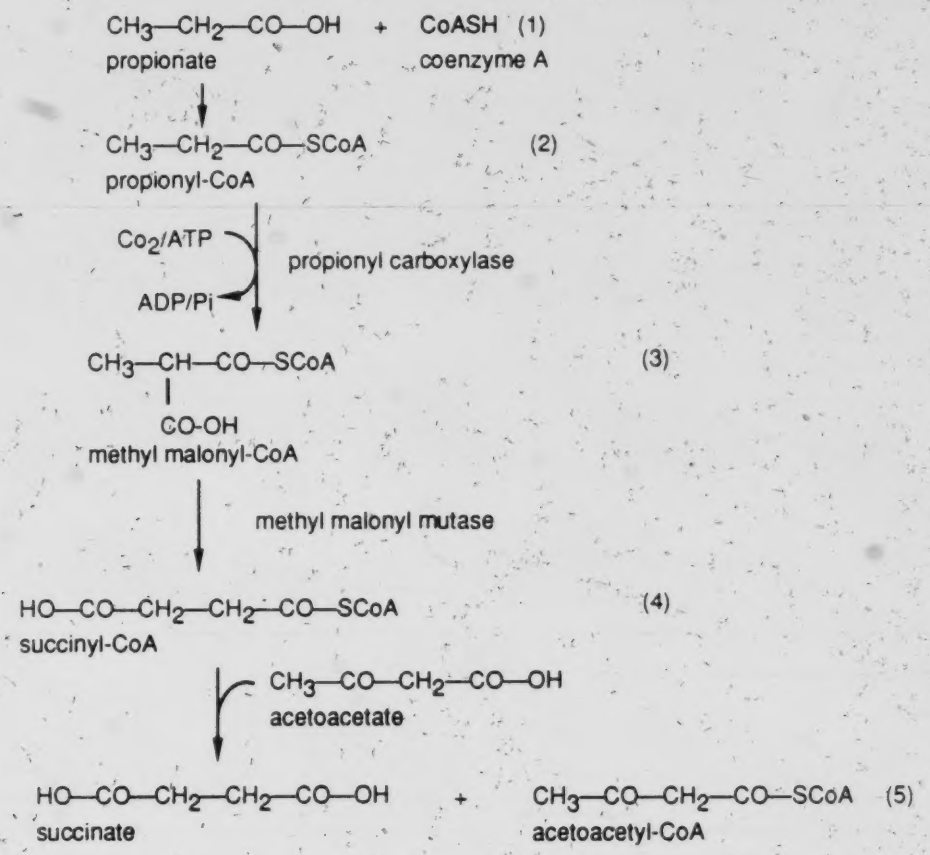


FIGURE 3

Formation of succinate from propionate



succinate. The ketone body acetoacetate is utilized in this reaction (Figure 3 (5)). This opposes the metabolism of even carbon chain fatty acids in which acetoacetate is a product (Figure 2 (9)). Since acetyl-CoA is generated from acetoacetate, the energetic utilization of propionate in the TCA cycle appears to be at the expense of energy utilization from ketone body sources. This is consistent with studies that have found propionate inhibitory to ketogenesis. Bush and Milligan (1971) illustrated the inhibition of ketogenesis upon addition of propionyl-CoA to bovine liver slices. The presence of succinate, formed in propionate metabolism, has been linked to enhanced gluconeogenesis, and has shown increases in liver glycogen when fed to rats (Scrutton and Utter, 1968).

6.4 THE EFFECT OF PROPIONATE ON SERUM LIPIDS

Propionate metabolism has been studied for a potential hypolipidemic effect (Thacker et al, 1981; Boila et al, 1981; Chen et al, 1984). Propionate has been shown to inhibit cholesterol synthesis rates in isolated rat hepatocytes (Anderson and Bridges, 1981). Lowered serum cholesterol was observed in rats consuming high cholesterol diets supplemented with propionate as compared to rats without propionate supplementation (Chen et al, 1984). Serum triglycerides were

not affected by propionate feeding although triglycerides were significantly raised by the high cholesterol diet.

Thacker and Bowland (1981) found that pigs fed hypercholesterolemic diets responded to 6 and 9% dietary propionate supplementation with reduced total plasma cholesterol and HDL cholesterol levels. No changes were observed in serum triglycerides. Feeding dietary propionate is not necessarily parallel to the absorption of propionate by the mucosa of the large intestine. It has been shown that SCFAs fed orally are absorbed in the stomach (Vahouny et al, 1988). This site of absorption makes propionate less available to the colonic mucosal cells and may decrease SCFAs reaching the liver (Argenzio and Southworth, 1974).

Inhibition of the enzyme 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase was shown by a 40% reduction in activity when bovine liver slices were incubated with butyrate and propionate as compared to incubation with butyrate alone (Bush and Milligan, 1971). Odd-number carbon chain fatty acids showed inhibition of cholesterol synthesis compared to even-number carbon chain fatty acids in rat liver homogenates (Wood and Migicovsky, 1956). The addition of succinyl-CoA to ox liver inhibited HMG-CoA synthase in the mitochondria in vitro (Lowe and Tubbs, 1985).

Of great interest is an alternate pathway that utilizes the ketone-body intermediate HMG-CoA (Figure 2 (8)) in cholesterol synthesis. The condensation reaction forming HMG-CoA is shown to be imperative in the synthesis of cholesterol (Lehninger, 1975). Figure 4 shows some of the steps involved in this pathway. If HMG-CoA synthase activity were reduced by the metabolism of propionate, HMG-CoA would not be as abundant for cholesterol synthesis.

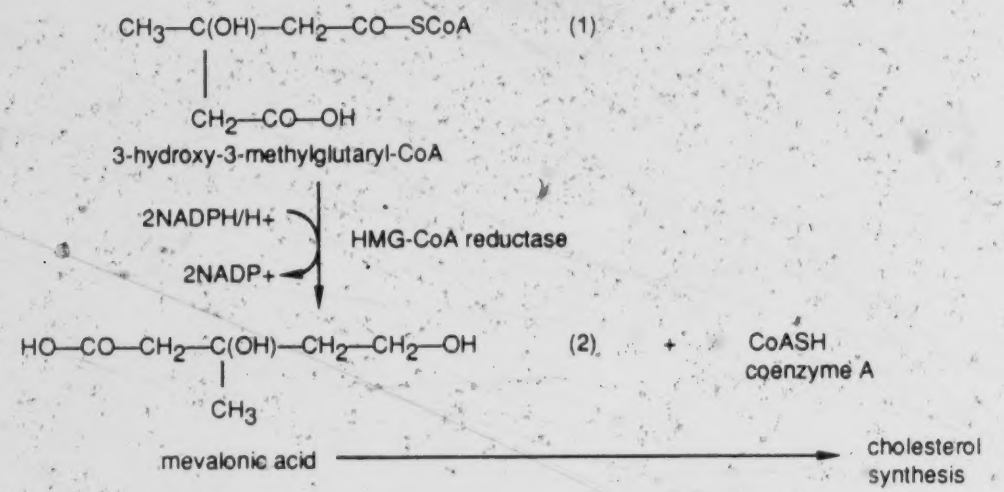
A reduction in cholesterol synthesis may explain results that find lowered blood cholesterol levels accompanying propionate ingestion (Thacker et al, 1981; Thacker and Bowland, 1981). A reduction in HMG-CoA reductase after pectin supplementation was observed by Proia et al (1981). HMG-CoA reductase is the rate limiting enzyme in cholesterol biosynthesis (Rodwell et al, 1976). A decrease in HMG-CoA synthase activity would provide less HMG-CoA as a substrate for the reductase (Figure 4 (1)) which may decrease the rate of cholesterol synthesis.

7 CHOICES OF AN ANIMAL MODEL

An appropriate model of human digestion is required for studies that are difficult to conduct with human subjects. The boredom of a homogeneous diet, the inconvenience to the subject of frequent sampling of blood and feces, the inability

FIGURE 4

Utilization of 3-hydroxy-3-methylglutaryl-CoA in formation of mevalonic acid for cholesterol synthesis



to employ surgical techniques, as well as the expense involved are a few reasons that make human studies more difficult than animal studies. Ileostomy patients are often used to study isolated upper intestinal effects of fiber supplementation (Bosaeus et al, 1986; Bosaeus and Andersson, 1987; McBurney et al, 1987). However, lower intestinal effects are difficult to isolate in human subjects. Many monogastric animals have been assessed for their usefulness as a model.

Non-human primates have the gastrointestinal physiology that most closely approaches that of humans (Kritchevsky et al, 1988). Vervet monkeys and baboons have been used in assessing blood lipids after fiber supplementation (Kritchevsky et al, 1974; Kritchevsky et al, 1981; Kritchevsky et al, 1986). A western diet high in saturated fat and cholesterol caused hypercholesterolemia in monkeys (Kritchevsky et al, 1986; Kritchevsky et al, 1988) showing similarities to humans. However, the expense involved and difficulties in maintenance of these animals prevent their widespread use.

The dog has a 7% energetic contribution from SCFA production (Stevens, 1977). This is comparable to the 5 - 10% contribution found in man (Bougat, 1987). However, the

carnivorous diet of the dog is too different from the human diet to use the dog as a model in human gastrointestinal function.

Rats are frequently used in the study of dietary fiber due to their inexpensive diet and maintenance. However, rats are primarily cecal fermenters (Graham and Aman, 1987) and are not recommended for dietary fiber studies (Van Soest et al, 1982). Rats have a colonic separation mechanism that enables them to form two types of fecal pellets, one which can be re-consumed and contains a higher protein and a lower fiber concentration than the other type (Sperber et al, 1983). Although coprophagy can be reduced by wire mesh cage bottoms, the influence of altered passage rate and corresponding changes in fermentation time on colonic SCFA production and absorption is unknown. The mean retention time of food is 6 - 10 hours in the rat. A shorter transit time yields fecal pellets high in protein and a longer retention time allows for enhanced fermentation of digesta fiber content. The human gut retention time of 30 - 40 hours provides no colonic separation mechanism. The rat may not be representative when a model is required to look specifically at colonic fermentation effects.

The pig is a practical and suitable choice as an animal model and has been shown to be similar to the human in

digestive physiology, size, and diet. The pig also displays similar metabolic responses to diet and disease as humans. The feeding of excess dietary cholesterol and fat to the adult pig can induce hypercholesterolemia (Mahley et al, 1975). A diet simulating the gross composition of a western diet for humans, providing 52, 30, and 18% of its energy as carbohydrate, fat, and protein, respectively, and supplemented with 1% crystalline cholesterol and 0.5% cholic acid, increased pig serum cholesterol to hypercholesterolemic levels of 8.4 mmol/litre (323 mg/dl) after a four week experimental period (Ahrens et al, 1986). Normal total serum cholesterol in pigs is about 2 mmol/litre (77 mg/dl) (Mahley et al, 1975). Grower diets supplemented with stabilized tallow to bring the total fat content of the diet to 1.% (w/w) resulted in cholesterol consumption of 0.06% (w/w) from the tallow (Thacker et al, 1981). Pigs consuming this diet for a four week period showed a 10% increase in serum lipids, attributable to the ingestion of cholesterol (Thacker et al, 1981).

7.1 PHYSIOLOGICAL SIMILARITIES BETWEEN HUMAN AND PORCINE GUT FUNCTION

Although the pig has a larger cecum than the human, it is still primarily considered a colonic fermenter (Graham and Aman, 1987). The relative volume of different compartments

of the gastrointestinal tracts of the pig and human are shown in Table 4 (Stevens, 1977).

Digesta retention time for the pig is 50 hours (Van Soest et al, 1982). Compared to humans, pigs may have more cpportunity to absorb SCFAs upon fermentation. It has been estimated that the pig can derive 15 to 30% of its basal energy requirement for maintenance from SCFAs produced from fermentation of fiber and absorbed by the gut (Friend et al, 1964; Kennelly et al, 1981; Rerat et al, 1985). The rate of uptake of SCFAs in the colon of the pig (8-10 umol/cm^2 per h) (Argenzio and Southworth, 1974) was found to be similar to that of humans (6-12 umol/cm^2 per h) (McNeil et al, 1978).

Variations in the ratio of acetate:propionate:butyrate exist between and within species (Bougat, 1987). Values of fecal SCFA ratios indicate that human and pig flora produce similar ratios, as shown in Table 5. Other ratios have been reported (Miller and Wolin, 1979; Kennelly et al, 1981; Roth et al, 1988). Using these molar ratios and adapting a previous equation (equation 1) for prediction of fermentation products, equation 4 shows molar SCFA production from fermentable carbohydrate in the pig. Equation 5 converts molar ratios to weight values of the components. Variations in colonic bacteria due to diet, climate, stress, illness, and

TABLE 4

Relative volume of gastrointestinal segments in the pig and human[1]

Compartment	Pig	Human
	Percent relative wet capacity	
Stomach	31	17
Small Intestine	27	66
Cecum	10	--
Colon	28	17
Rectum	4	--

[1] Adapted from Stevens, 1977

TABLE 5

Ratios of short chain fatty acids found in the feces of humans and pigs

	Molar ratios of acids (%)			
	Acetate	Propionate	Butyrate	Reference
Pig	69	23	8	Sambrook, 1979
Human	60	24	16	Cummings, 1981

heredity can affect ratio and quantity of SCFAs formed (Holdeman et al, 1976).

$$326.5\ C_6H_{12}O_6 \text{ -----> } 414 \text{ acetate} + 138 \text{ propionate} + 48 \text{ butyrate} + 204\ CH_4 + 321\ CO_2 + 117\ H_2O \quad (4)$$

$$100g\ C_6H_{12}O_6 \text{ --------> } 42.3g \text{ acetate (705 mmol)} + 17.4g \text{ propionate (235 mmol)} + 7.2g \text{ butyrate (82 mmol)} + 5.6g\ CH_4 + 24.0g\ CO_2 + 3.6g\ H_2O \quad (5)$$

Some differences exist between the fecal flora of pigs and humans. The most commonly occuring colonic microbes in both the porcine colon and the human colon are listed in Table 2. The largest difference is the greater presence of cellulolytic bacteria in pig than in human fecal flora (Varel et al, 1984). This would enable pigs to more efficiently utilize cellulosic fiber fractions which may partially account for the greater percentage of metabolic energy from colonic fermentation seen in pigs as compared to humans (Bougat, 1987).

Studies that have looked at colonic bacterial population of both the human (Holdeman et al, 1976) and the pig (Robinson et al, 1981) have observed more variation between different

subjects than for a single subject after a change in diet. Changes in human fecal flora have been observed during stressful situations such as confinement to isolated quarters or prior to writing an examination (Holdeman et al, 1976). Changes in transit time have also been observed in stressful situations with pigs (Fleming and Acre, 1986). The potential for changes due to a stressful situation is of concern when working with animals. Sufficient time for adaptation to an experimental situation is required to avoid confounding results in fermentation studies.

7.2 SIMILARITIES BETWEEN HUMAN AND PORCINE LIPOPROTEINS

Lipoprotein densities in pig serum are in general agreement in the literature. The LDL fraction is found mainly between densities of 1.03 and 1.08 g/ml (Mahley and Weisgraber, 1974), with the main components concentrated between 1.063 and 1.074 g/ml (Fidge and Smith, 1975). The main HDL fraction is found between densities of 1.09 and 1.21 g/ml (Nestruck et al, 1977), although some HDL has been found at densities as low as 1.07 g/ml. (Mahley and Weisgraber, 1974). VLDL, although considered a minor component of pig serum (Mills and Taylaur, 1971) is generally isolated at densities below 1.03 g/ml (Mahley and Weisgraber, 1974).

Human lipoprotein separation by density defines lipoproteins present in the serum fraction of density less than 1.019 g/ml as VLDL. Lipoproteins present in the fraction between densities 1.019 and 1.063 g/ml are LDL, and those present in the faction of density greater 1.063 g/ml are HDL (Havel et al, 1955).

Miniature swine fed 15% lard and 1.5% cholesterol for 7 months had an increase in serum cholesterol of 400% over controls (Mahley et al, 1975). This model may not be representative of the hyperlipidemic human. These pigs showed altered lipoprotein distribution in the lower density fractions. In control pigs 64% of serum cholesterol was contained in lipoproteins of densities less than 1.06 g/ml. Hyperlipidemic pigs had 90% of cholesterol in lipoproteins of densities less than 1.06 g/ml. However, the hyperlipidemic swine showed an arginine-rich B-apoprotein in VLDL that co-electrophoresed with B-VLDL from type III hyperlipidemic humans (Mahley et al, 1975). In normal pigs HDL has 1/4 as much triglyceride and twice as much cholesterol ester as human HDL (Mills and Taylaur, 1971). Pig HDL is a more homogeneous protein than human HDL (Chapman, 1980). As differences do exist between human and porcine lipoproteins, saturated fat and cholesterol feeding studies using the pig as a model of the human should be interpreted with caution.

7.3 THE CANNULATED PIG MODEL

Cannulation at the distal ileum is a technique that can be used in animal models to monitor upper intestinal and lower intestinal effects separately. A T-cannula fitted at the distal ileum, adapted from duodenal cannulation as described by Sauer et al (1983), enables digesta sampling. Upper intestinal nutrient digestibility can be determined. Cannulation has been successfully employed in pigs by other researchers (Bjornhag and Jonsson, 1984; Fleming and Wasilewski, 1984; Graham et al, 1985; Aherns et al, 1986; Kik et al, 1988; vanLeeuwen et al, 1988; deLange et al, 1989a).

Infusion of propionate through a cannula at the cecum would be a useful model in assessing the effect of colonically derived propionate independent of upper intestinal effects of fiber supplementation. Energetic utilization of intracecally infused propionate in sows has shown complete propionate utilization when 31.4% of the animal's daily energy was infused through a cannula as propionate (Roth et al, 1988).

HYPOTHESIS

The dietary supplementation of fermentable fibers has displayed more of an effect on serum lipid lowering than supplementation of non-fermentable fibers. The enhanced formation and absorption of propionate as an end product of fiber fermentation may be responsible in part for the hypocholesterolemic effect of water-soluble fiber supplementation. Propionate may inhibit hepatic HMG-CoA synthase, an enzyme required for cholesterol synthesis. Cecal propionate infusion in a hypercholesterolemic cannulated pig model may decrease hepatic cholesterol synthesis and result in lowered serum cholesterol.

METHODOLOGY

1 ANIMALS

Ten York-Landrace barrows weighing between 27 kg and 40 kg (average 34.5 kg) were obtained from the Swine Unit at the University of Alberta Research Farm. Animals were housed individually in metabolic crates in the Metabolic Barn at the University of Alberta Research Farm. All aspects of the experiment were approved by the University of Alberta Animal Policy and Welfare Committee.

2 CANNULATION

2.1 SURGERY

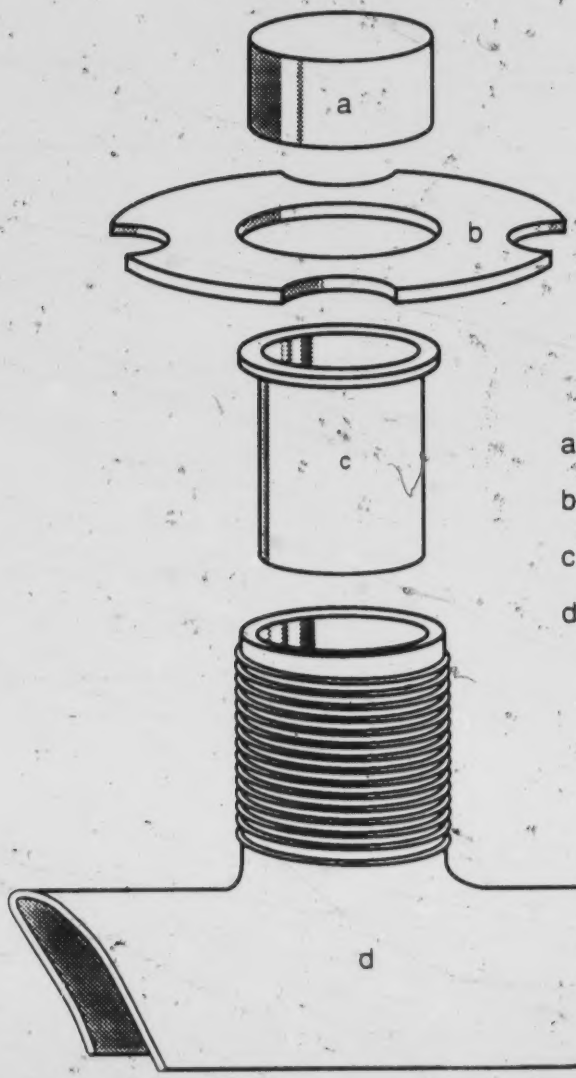
Animals were fasted 24 hours prior to surgery. Surgery was performed under standard sterile surgical conditions at the University of Alberta Research Farm. Pigs were fasted for 24 hours prior to surgery, and given a tranquilizer (2 ml Atravet, 25 mg/ml acepromazine maleate, Ayerst) at least 30 minutes prior to surgery. Halothane (2% at 4 litres/minute in 1:3 $N_2O:O_2$) was used to anesthetize animals. The cannulation was performed as described by Sauer (PhD Thesis, 1976), with the following modifications.

A T-cannula (Figure 5) was inserted at the distal ileum approximately 10 cm proximal to the cecum. A smaller cannula (Figure 6) was made from a cylindrical L-shaped mold which was repeatedly dipped in liquid polyvinylchloride plastisol and hardened by baking for 15 seconds at 400°C after each dip. A purse string suture was placed on the dorsal portion of the cecum and the cecum was punctured. The smaller (cecal) cannula was inserted into the cecum and the purse string suture was tightened and tied. A second purse string suture was placed around the cannula.

Terramycin (Phizer) was placed in the incision cavity (2 ml of 100 mg/ml) before closing. Both cannulae were exteriorized on the right side of the pig. The T-cannula had a screw-cap that could be removed for collection of ileal effluent. The cecal cannula had a butyl rubber stopper (approx 1.5 cm diameter) to prevent leakage. A hole was drilled in the stopper to allow for the insertion of 25 cm of tygon tubing (Fisher Scientific, formulation R-3603, 1.5 mm ID, 3.0 mm OD) through the cannula and into the cecum. The tubing, present inside the cecum, had been previously perforated with 30 holes evenly distributed along the last 15 cm of its length. Tygon tubing (20 cm) extended through the stopper in the cecal cannula, exterior to the pig. A needle (Beckton-Dickinson 18 G 1 1/2) with the sharp tip removed was inserted into the exterior end of the tygon tubing. An

FIGURE 5

The distal ileal T-cannula



a - screw cap

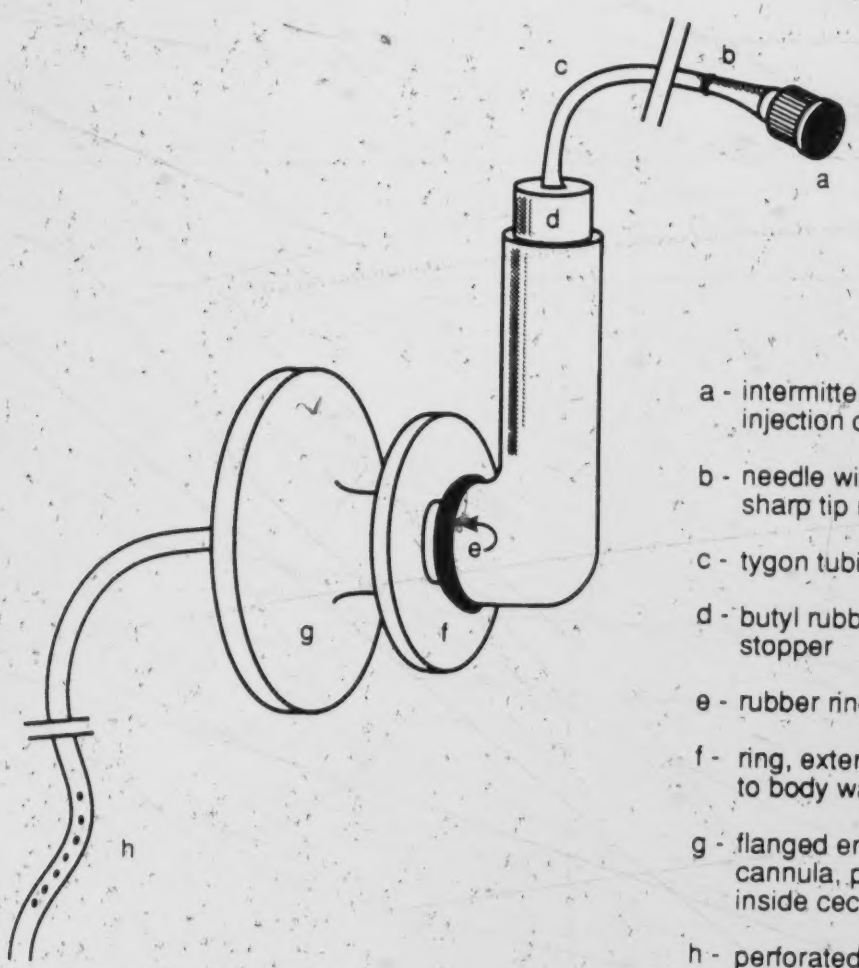
b - ring, exterior to body wall

c - central cylinder

d - flanged end of cannula, placed inside terminal ileum

FIGURE 6

The cecal infusion cannula



a - intermittent injection cap

b - needle with sharp tip removed

c - tygon tubing

d - butyl rubber stopper

e - rubber ring

f - ring, exterior to body wall

g - flanged end of cannula, placed inside cecum

h - perforated tygon tubing

intermittent injection cap (Short INT Stopper, Luer Lock, Burron Medical Inc.) was screwed onto the needle so that the cecal infusion line could be attached once the animal was in the metabolic crate.

2.2 RECOVERY

Pigs were returned to their metabolic crates immediately after surgery. One animal did not survive the post-surgical period. Terramycin (Animal Formula Soluble Powder, Kane Veterinary Supplies, Edmonton, Alta) was administered orally (10 mg/kg body weight per day) for three days post-surgery. Motrin (Upjohn) was administered orally (200 - 400 mg, 1 - 3 times/day) for pain relief as necessary. Pigs consumed between 100 g and 300 g of a standard grower diet (see section 4.1) twice daily during the first four days of the post-surgical recovery period.

3 EXPERIMENTAL DESIGN

The experiment was planned as a crossover design in which each pig would receive saline infusion (control) and propionate infusion. Ten pigs were cannulated so that at least eight would be available to provide an adequate sample size. The nine pigs that survived surgery were assigned to one of two groups such that the average animal weights were

not different between infusion groups. Five pigs received propionate infusion in the first period and saline infusion in the second period. The other four pigs received infusion treatments in the reverse order. A time line of the experimental design is shown in Figure 7.

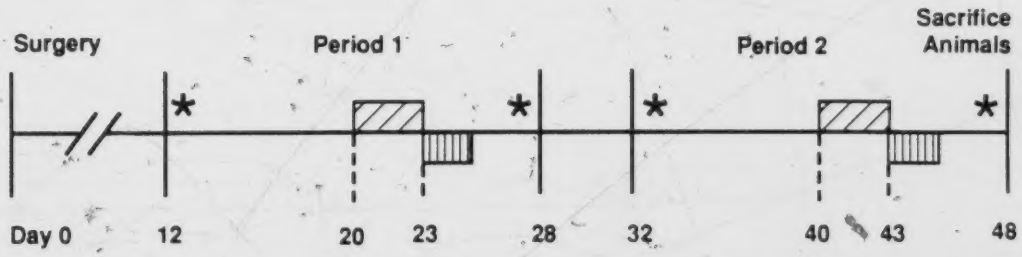
3.1 INFUSION PERIODS

The first infusion period began 12 days after the last surgery. An Ismatec MP-13 (Brinkman Instruments, Rexdale, Ontario) peristaltic infusion pump was used to provide a flow rate of 1.05 ml/minute (1512 ml/day). Tygon tubing (Fisher Scientific, formulation R-3603, 1.5 mm ID, 3.0 mm OD) was from the infusion pump and connected to a needle (Beckton-Dickinson 18 G 1 1/2), with the sharp tip removed, that punctured the intermittent injection cap exterior to the cecal cannula.

Infusate was individually supplied to each infusion line from a stoppered 2 litre nalgene bottle. A hole was drilled in the stopper to allow for the insertion of the tygon tubing infusion line into the nalgene bottle. A needle (Beckton-Dickinson 18 G 1 1/2) also punctured the stopper to allow air into the bottle to replace the infusate. Infusion lines were weighted to prevent the lines from floating and drawing in

FIGURE 7

Study design



* Blood sampling

72h fecal collection

48h digesta collection

- 9 pigs in a crossover design
- Infusion at 1.05 ml/min
  - Saline = 0.9% NaCl
  - Propionate = 36.4 mmol/kg$^{0.75}$ per day
    Period 1 - 44.4 g/day
    Period 2 - 56.7 g/day

air. Infusion was continuous during the two 16 day periods. A four day non-infusion period occurred between the two infusion periods.

3.1.1 SALINE INFUSION

Physiological saline (0.9% NaCl w/w) was prepared with distilled water every second day. Pigs received 13.6 g NaCl/day into the cecum during both the first and second saline infusion periods.

3.1.2 PROPIONATE INFUSION

Sodium propionate was prepared with distilled water every second day. The sodium salt was formed by adding NaOH and propionate in equimolar quantities. Pigs received 36.4 mmol propionate/kg of metabolic body weight ($kg^{0.75}$) per day. Metabolic body weight was calculated at the beginning of each infusion period based on the average weight of all nine pigs. During the first infusion period, this corresponded to 600 mmol propionate/day (44.4 g propionate/day or 58.2 g sodium propionate/day) providing 219 kcal/day. During the second infusion period, pigs received 756 mmol propionate/day (56.7 g propionate/day or 73.3 g sodium propionate/day) providing 276 kcal/day. The infusate concentrations were 397 mmol propionate/litre and 500 mmol propionate/litre in periods 1

and 2% respectively. Sodium propionate was present in the infusate solutions at a level of 3.85% (w/w) in period 1 and 4.85% (w/w) in period 2. This represented 3.5% extra kcal/day in the form of propionate.

3.2 COLLECTIONS

3.2.1 ILEAL DIGESTA COLLECTION

Digesta collections were conducted on the 12th and 13th days of each infusion period. The T-cannula was used to obtain a sample of the digesta passing from the distal ileum into the colon. The screw cap was removed from the cannula, and 2 m lengths of 5 cm flat polyethylene tubing bags were secured to the opening of the cannula with an elasticized velcro fastener. The closed end of the plastic tubing was placed in a bucket of ice water to ensure that effluent collected was kept close to 0°C to minimize bacterial and enzymatic degradation. After the three hour collection period, effluent was stored at -25°C.

The three hour collection periods were followed by three hour non-collection periods to minimize the daily loss of effluent to the large bowel during collection. Collection periods for the 12th day of each infusion period began at 12 midnight, 6 am, 12 noon, 6 pm. Collection periods for the 13th

day of each infusion period began at 3 am, 9 am, 3 pm, and 9 pm. The 24 hours of collection were divided out over the two days such that the cumulative hours of collection represented every hour of the day. The digesta collected from a pig during all eight of the three hour collection periods was weighed, freeze-dried, pooled, and weighed again. Freeze-dried digesta was ground to a fine consistency in a grinder mill (Kurzzeitbetrieb Micro Mill, Terochem), and stored at 23°C for later analysis.

3.2.2 FECAL COLLECTION

A seventy-two hour fecal collection period occurred on days 9, 10, and 11 of each infusion period. Feces were collected three times daily and weighed. Water was added as 10% of the fecal weight to facilitate homogenization. The fecal collection with added water was homogenized by pummelling it in heavy plastic zip-lock bags. A 10% aliquot of the resulting fecal homogenate was stored at -30°C. Later, these aliquots were freeze-dried, weighed, ground to a fine consistency in a grinder mill, and stored at 23°C until further analysis.

3.2.3 BLOOD COLLECTION

On days 1 and 17 of each infusion period, blood was drawn from each animal. Serum collection Vacutainers (Beckton Dickinson, 10 ml or 15 ml tubes) were used to collect blood through jugular puncture. Tubes were kept on ice for no more than one hour prior to centrifugation. Centrifugation occurred at 3000 rpm for 30 minutes at 4°C on a Beckman J-6M/E Centrifuge. Serum was removed and stored at -30°C for two to three months prior to analysis.

3.2.4 LIVER COLLECTION

After the 8 am feeding on the 17th day of the second infusion period, pigs were sacrificed and the livers removed. Livers were immediately weighed and kept at 4°C for four hours. Liver samples were then removed with a cork bore and stored at -30°C for two months prior to analysis.

4 DIETS

4.1 CONSUMPTION

Pigs consumed between 100 and 300 g (323 - 969 kcal) of grower diet twice daily at 8 am and 6 pm during the first 4 days of the post-surgical recovery period. This intake was

gradually increased to 900 g (2907 kcal) twice daily. Once all pigs were consuming 1800 g/day (5814 kcal/day) of the grower diet, animals were switched to the high fat diet.

Pigs consumed the high fat diet for at least five days prior to the start of the first infusion period to ensure adaptation to the diet. Pigs were fed 109.3 g/$kg^{0.75}$ per day (376 kcal/$kg^{0.75}$ per day) in both infusion periods based on the average weight of all pigs on the first day of the infusion period. This amounted to 1800 g/day (6194 kcal/day) in the first infusion period, and 2268 g/day (7804 kcal/day) in the second infusion period. Feed intakes were adjusted according to metabolic body weight to ensure that any differences found between the two infusion periods would not be due to a decreased energy intake per metabolic body weight in the second infusion period. Pigs were fed two equal meals at 8 am and 6 pm throughout the experiment.

4.2 COMPOSITION

Formulations of the standard grower and high fat diets are given in Table 6. Nutrient contents of both diets were calculated from nutrient composition tables and are given in Table 7.

TABLE 6

Formulation of standard grower diet and high fat diet

Component	Percent of diet (by weight)	
	Grower	High Fat
Wheat	25.00	22.44
Barley	53.10	47.88
Soybean meal	16.00	18.95
Tallow	2.00	6.38
Iodized salt	0.40	0.40
Biofos	1.50	1.50
Limestone (33% Ca)	1.00	1.00
Vitamin/mineral premix*	1.00	1.20
Chromic oxide	-	0.25
Total	100.00	100.00

* Vitamin/mineral premix contains the following per 10 g: 120 mg Zn, 48 mg Mn, 100 mg Fe, 10 mg Cu, 0.1 mg Se, 7500 IU vit A, 700 IU vit D, 45 IU vit E, 12 mg riboflavin, 40 mg niacin, 27 mg Ca-pantothenate, 28 ug vit B_{12}.

TABLE 7

Nutrient content of selected nutrients in the grower diet and the high fat diet[1]

Nutrient			Grower	High Fat
Energy[2]	DE	(kcal/kg)	3230	3441
	ME	(kcal/kg)	3112	3310
Protein[3]		(g/kg)	163	167
Fat[4]		(g/kg)	34.8	77.6
	SFA	(g/kg)	10.3	26.2
	PUFA	(g/kg)	10.8	17.0
	PUFA/SFA		1.05	0.65
Cholesterol[5]		(mg/kg)	105	336
Ca		(mg/kg)	4173	4229
Fe		(mg/kg)	170	189
Zn		(mg/kg)	146	170
Mn		(mg/kg)	48.0	57.6
Cu		(mg/kg)	10	12
Se		(mg/kg)	0.10	0.12
Vitamin A		(IU/kg)	7500	9000
Vitamin D		(IU/kg)	700	840
Vitamin E		(IU/kg)	45	54
Vitamin B_{12}		(ug/kg)	28.0	33.6
Niacin		(mg/kg)	93.1	101.6
Riboflavin		(mg/kg)	13.6	16.0
Thiamin		(mg/kg)	4.47	4.30

[1] Calculated from values found in National Research Council's Nutrient Requirements of Swine, 1988.
[2] Energy calculated as digestible energy
[3] Protein calculated as crude protein
[4] Fat calculated as ether extract
[5] Cholesterol analysed as outlined under 4.3.6

Diets were mixed at the University of Alberta Research Farm feed mill. Both diets were ground to a fine meal consistency, to prevent clogging or irritation by large particles at cannulation sites. Two batches of high fat diet were mixed. One batch was fed during the first infusion period, and the other during the second infusion period. Four aliquots (200 g) of feed were randomly sampled from each batch of feed for subsequent analysis.

4.3 LABORATORY ANALYSIS OF DIET

4.3.1 MOISTURE

Moisture content of the high fat diet was determined as a percentage of initial feed weight. Empty porcelain crucibles were dried overnight at 85°C and hot weighed. Feed samples (1.0 - 2.0 g) were weighed into cooled crucibles and dried in a Precision Scientific convection oven at 100°C for 8 hours. The dry feed samples were hot weighed to reduce moisture absorption while cooling. The moisture content of the 4 feed samples from both batches of high fat diet was analysed in duplicate.

4.3.2 ASH

Ash content of the high fat diet was analysed according to the AOAC (1984) method for determination of ash in animal feed and expressed as a percentage of initial feed weight. Empty porcelain crucibles were dried overnight at 100°C and hot weighed. Feed samples (3.0 - 4.0 g) were weighed into the cooled crucibles, and ashed in a Lindberg Hevi Duty muffle furnace oven at 550°C for 24 hours. Crucibles were then placed in a convection oven at 100°C for at least 4 hours before hot weights were obtained. The ash content of the 4 feed samples drawn from both batches of high fat diet was analysed in duplicate.

4.3.3 CHROMIC OXIDE

Chromic oxide was added to the high fat diet at a level of 0.25% by weight. Cr_2O_3 passes through the digestive tract unabsorbed, and can be used as a digestibility marker. The concentration of Cr_2O_3 to components in feces and ileal digesta was compared to the concentration of Cr_2O_3 to components in the diet to determine the digestibility of the components.

Chromic oxide content of the high fat diet was analysed using sodium peroxide fusion (Calvert et al, 1989). Feed samples (3.0 - 4.0 g) were weighed into 30 ml porcelain

crucibles and heated in a Lindberg Hevi Duty muffle furnace oven at 550°C for 48 hours. Crucibles were cooled and 2.0 - 3.0 g of Na_2O_2 were mixed in with the ash. This mixture was covered with an additional 2.0 - 3.0 g Na_2O_2. Crucibles were covered and heated to 700°C in a muffle furnace oven for 25 minutes. After cooling for two hours, crucibles were placed in 400 ml beakers and placed under a fume hood. Crucibles were filled about two-thirds full with distilled water, and left to stand for ten minutes while oxygen and alkaline fumes were released.

The contents of each crucible was then rinsed into a beaker with 50 - 100 ml of distilled water. Glass rods were used to scrape off the material adhering to the sides of the crucibles. Crucibles were rinsed with a 1:8 (vol:vol) solution of sulfuric acid and distilled water which was allowed to remain in the crucibles for 3 - 5 min to free any remaining Cr-containing residue. These solutions were added to the contents of the beakers, and crucibles were rinsed with 10 ml distilled water. The pH of the resulting mixture (11.5 - 12.5) was adjusted to 8.5 - 9.5 with concentrated H_2SO_4.

The content of each beaker was transferred quantitatively into 250 ml volumetric flasks and brought to volume with

distilled water. Flasks were agitated thoroughly, and left to stand at 23°C overnight to allow for settling of insoluble material.

A stock solution of potassium dichromate containing 3.735 g K_2CrO_4 in one litre of distilled water (1 mg Cr/ml) was prepared. Subsequent dilutions of this stock solution were made to prepare standards containing between 0.5 and 10.0 mg Cr/litre. Aliquots (1.0 ml) of supernatant from the 250 ml volumetric flask were mixed with water (5.0 ml), and absorbance was read at 366 nm on a Unicam SP1800 Ultraviolet Spectrophotometer. Distilled water was used for a blank. Standards were measured with each set of samples to determine sample concentrations. The chromium content of the 4 feed samples from both batches of high fat diet was analysed in triplicate and expressed as mg Cr_2O_3/g feed.

4.3.4 NITROGEN

Nitrogen content of the high fat diet was analysed according to the AOAC (1984) Kjeldahl method using a Micro-Kjeldahl system. Nitrogen present in a sample is digested in strong sulfuric acid with a mercuric catalyst and converted

to $(NH_4)_2SO_4$. In the presence of NaOH, the ammonia salt is distilled as NH_3 into a weak acid-base indicator solution and titrated to end-point with a weak acid.

The Micro-Kjeldahl system used consisted of a Kjeltec Digestion System 20 1015 Digester and a Kjeltec System 1002 Distilling Unit. Feed samples (1.0 - 2.0 g) were weighed into Kjeldahl digestion tubes. One Kjel-Tab (3.5 g K_2SO_4, 0.175 g HgO) and 12.5 ml of reagent grade H_2SO_4 were added to each digestion tube. Tubes were digested for 4 hours to ensure complete digestion. Distilled water (75 ml) was added to each tube when digestion was complete. An aliquot (110 ml) of 40% NaOH containing 60 g/litre sodium thiosulfate was dispensed to each tube prior to distillation. Distillates were collected in 250 ml Erlenmeyer flasks containing 25 ml of a solution of 4% (w/w) boric acid with methyl red and bromcresol green indicators. Flasks were titrated with 0.1 N H_2SO_4 to endpoint. Standards and blanks were prepared by digesting and distilling $(NH_4)_2SO_4$ and water, respectively.

The nitrogen content of the 4 feed samples from both batches of the high fat diet was analysed in quadruplicate. The concentration of nitrogen in the diet was expressed as mg N/g feed and as mg N/mg Cr_2O_3.

4.3.5 ORGANIC MATTER

Organic matter in the high fat diet was calculated as the weight remaining when water and ash weights were subtracted. This was calculated for each of the 4 feed samples from both batches of the high fat diet and expressed as a percentage of initial feed weight, as shown in equation 6, and as g organic matter/mg Cr_2O_3.

$$\% \text{ ORGANIC MATTER} = 100\% - (\% \text{ WATER} + \% \text{ ASH}) \quad (6)$$

4.3.6 CHOLESTEROL

The total cholesterol content of the high fat diet was analysed using a colorimetric method Test-Combination kit from Boehringer Mannheim (Cat. no. 139-050). To determine total cholesterol, a methylation procedure was used to free esterified cholesterol.

Cholesterol was quantitated as follows. Cholesterol is converted to delta-4-cholestenone and H_2O_2 in the presence of cholesterol oxidase and oxygen. The enzyme catalase converts methanol to formaldehyde and water in the presence of H_2O_2. Formaldehyde reacts with ammonium ion and 2 acetylacetone molecules to form 3,5-diacetyl-1,4-dihydrolutidine or lutidine-dye. This dye is a yellow compound that can be

detected by visible light absorption at 405 nm. The concentration of dye molecules formed is proportional to the concentration of cholesterol present. Cr_2O_3 present in the diet does not absorb at 405 nm.

4.3.6.1 EXTRACTION AND METHYLATION

A feed sample (1.0 - 2.0 g) was placed in a 100 ml round-bottom flask with 1 g sea sand and 10 ml of a freshly prepared methanolic KOH (1.0 mol/litre) solution. The flask was boiled in a heated sand bath under reflux for 30 minutes. the contents were stirred with a magnetic stir bar. The supernatant was removed and placed in a 50 ml volumetric flask. The residue was boiled twice with 10 ml volumes of isopropanol for five minutes under a reflux condenser with stirring. The supernatant was added to the 50 ml volumetric flask. The contents of the flask were cooled to 23°C, made up to the mark with isopropanol and agitated thoroughly. The contents of the flask were filtered through a fluted paper filter (Whatman Qualitative Filter Paper No.3, 9.0 cm) into a capped 100 ml bottle. This was the sample solution.

4.3.6.2 ENZYMATIC ASSAY FOR CHOLESTEROL DETERMINATION

A reaction mixture containing methanol (1.71 mmol/ml), acetylacetone (0.019 mmol/ml), catalase (1420 U/ml), ammonium

phosphate buffer (pH 7.0), and stabilizers was prepared. The reaction mixture (4.0 ml), distilled water (1.0 ml), and the sample solution (0.4 ml) were placed into 10 ml borosilicate glass disposable test tubes. The tubes were mixed thoroughly and a 2.5 ml aliquot of this solution was placed in a second test tube. The first tube was used as a sample blank. Cholesterol oxidase (0.02 ml, 15 U/ml) was added to the second tube (sample reaction tube). A standard solution of cholesterol in isopropanol (1.0 mg/ml) was used to prepare standards. Standards were prepared for each set of tubes measured. Isopropanol was used for the standard blank. All tubes were incubated in a 37°C water bath for 60 min.

After cooling to 23°C, the absorbance at 405 nm of both the sample blank and the sample reaction tube were recorded against air. The difference in absorbance at 405 nm between the sample blank and the sample reaction tube (delta A) was compared to the delta A of standards to quantitate cholesterol concentrations of samples. The cholesterol content of the 4 feed samples from both batches of the high fat diet was analysed in duplicate and expressed as mg cholesterol/g feed and mg cholesterol/mg Cr_2O_3.

5 ILEAL DIGESTA AND FECAL ANALYSIS

5.1 MOISTURE

Freeze-dried ileal digesta and fecal homogenate moisture contents were analysed as outlined under 4.3.1. Moisture was expressed as a percentage of initial and freeze-dried weight. The moisture content of the 24 hour digesta samples and 72 hour fecal samples for each pig from both infusion periods was analysed in duplicate.

5.2 ASH

Freeze-dried ileal digesta and fecal homogenate ash contents were analysed according to the AOAC (1984) method outlined under 4.3.2. Digesta and fecal samples (1.0 - 2.0 g) were analysed in duplicate for each pig from both infusion periods. Ash was expressed as a percentage of initial and freeze-dried weight and mg ash/mg Cr_2O_3.

5.3 CHROMIC OXIDE

Cr_2O_3 content of freeze-dried ileal digesta and fecal homogenate was analysed using sodium peroxide fusion (Calvert et al, 1989) as outlined under 4.3.3. Samples (1.0 -2.0 g)

were analysed in duplicate. Cr_2O_3 was expressed as mg Cr_2O_3/g freeze-dried sample.

5.4 NITROGEN

Freeze-dried ileal digesta and fecal homogenate nitrogen contents were analysed according to the AOAC (1984) Kjeldahl method as outlined under 4.3.4. Samples from both infusion periods (1.0 - 2.0 g) were analysed in quadruplicate for each pig. Nitrogen content was expressed as mg N/mg Cr_2O_3. Nitrogen digestibilities were calculated as shown in equations 7 and 8, and expressed as a percentage.

$$\% \text{ ILEAL NDIG} = ((\text{FDN} - \text{DGN})/\text{FDN}) \times 100 \quad (7)$$

where FDN = mg N/mg Cr_2O_3 in feed

DGN = mg N/mg Cr_2O_3 in digesta

NDIG = nitrogen digestibility

$$\% \text{ FECAL NDIG} = ((\text{FDN} - \text{FCLN})/\text{FDN}) \times 100 \quad (8)$$

where FDN = mg N/mg Cr_2O_3 in feed

FCLN = mg N/mg Cr_2O_3 in feces

NDIG = nitrogen digestibility

5.5 ORGANIC MATTER

Freeze-dried digesta and fecal homogenate organic matter contents were calculated as the weight remaining when freeze-dried water and ash weights were subtracted, as outlined under 4.3.5. This was calculated for each pig during each infusion period. Organic matter was expressed as a percentage of freeze-dried sample weight and as g organic matter/mg Cr_2O_3. Ileal and fecal organic matter digestibilities were calculated as shown in equations 9 and 10, and expressed as a percentage.

$$\% \text{ ILEAL OM DIGESTIBILITY} = ((FDO - DGO)/FDO) \times 100 \quad (9)$$

where FDO = g OM/mg Cr_2O_3 in feed

DGO = g OM/mg Cr_2O_3 in digesta

OM = organic matter

$$\% \text{ FECAL OM DIGESTIBILITY} = ((FDO - FCLO)/FDO) \times 100 \quad (10)$$

where FDO = g OM/mg Cr_2O_3 in feed

FCLO = g OM/mg Cr_2O_3 in feces

OM = organic matter

5.6 CHOLESTEROL

Freeze-dried ileal digesta and fecal homogenate cholesterol contents were analysed using the colorimetric method Test-Combination kit from Boehringer Mannheim (Cat. no.

139-050) as outlined under 4.3.6 with the following modifications.

Freeze-dried samples (1.0 - 2.0 g) were placed in 50 ml teflon-lined screw-capped methylation tubes with 1 g sea sand and 10 ml of a freshly prepared methanolic KOH (1.0 mol/litre) solution. The tubes were agitated thoroughly and placed for 60 minutes in a sand bath heated to 100°C on a hot plate. Tubes were cooled slightly, the supernatants were removed and placed in 50 ml volumetric flasks. The residues were boiled twice with 10 ml volumes of isopropanol for 10 minutes in the capped tubes placed in the sand bath. The supernatants were added to the 50 ml volumetric flasks. The contents of the flasks were cooled to 23°C, made up to the mark with isopropanol and agitated thoroughly. The contents of the flasks were filtered through a fluted paper filter (Whatman Qualitative Filter Paper No.3, 9.0 cm) into a capped 100 ml bottle. This was the sample solution.

The sample solutions were analysed as outlined under 4.3.6.2. Samples were analysed in duplicate. Cholesterol content was expressed as mg cholesterol/mg Cr_2O_3.

5.7 BILE ACID

Bile acid analysis was carried out based on an enzymatic method (Sheltawy and Losowsky, 1975) with modification (Gallaher and Schneeman, 1986). Freeze-dried ileal digesta and fecal homogenate bile acid contents were analysed in duplicate and expressed as nmol bile acid/mg Cr_2O_3.

The enzyme 3-alpha-hydroxy steroid dehydrogenase (3-alpha-HSD) is an enzyme specific for the oxidation of the 3-alpha-hydroxyl group of C-24 steroids, found in 90% of fecal bile acids. When this oxidation occurs in the presence of NAD, NADH is formed and detected at 340 nm. NADH formation is stoichiometric with the amount of 3-alpha-hydroxy bile acid present.

5.7.1 EXTRACTION

Ground, freeze-dried samples (0.3 - 0.7 g) with 0.5 N HCl in absolute ethanol (5 ml) were agitated for 30 sec in screw-cap 15 ml test tubes . Tubes were then incubated for 1 hour at 37°C with agitation every 15 min. Tubes were centrifuged at 1000 x g for 10 minutes at 22°C.

The supernatant was transferred to a weighed 10 ml screw-cap test tube. The pellet was again extracted with 0.5 N HCl

in absolute ethanol (5 ml), then incubated, agitated, and centrifuged as above. The two supernatants were pooled and dried under partial vacuum at 25°C. The dried extract was refrigerated at 4°C until analysis.

The dried extract was resuspended with methanol (0.6 ml) and mixed with a vortex mixer for 15 sec. Distilled water (2.4 ml) was then added and the mixture vortexed. This was the extraction suspension. The tube was weighed and the empty tube weight subtracted to determine the weight of the extraction suspension. The density of this solution was assessed as 1 mg/ml and the volume was calculated.

5.7.2 REMOVAL OF LIPIDS FROM EXTRACT

A Waters Sep-Pak C18 Reverse-Phase cartridge was attached to a 10 ml Beckton Dickinson disposable syringe. The cartridge was washed with methanol (10 ml) followed by distilled water (10 ml). Solvents were eluted by gravity. An aliquot of the extraction suspension (1.0 ml) was then loaded onto the cartridge and allowed to sink in.

The cartridge was then washed with distilled water (5.0 ml), hexane (5.0 ml), distilled water (5.0 ml), and 40:60 methanol:water (5.0 ml). All of the effluent was discarded. A solution of 75:25 methanol:water (4.0 ml) was then

introduced onto the cartridge and the effluent was collected in a weighed borosilicate glass disposable 15 ml test tube. Two subsequent 3 ml volumes were introduced onto the column and collected in the same tube. The tube containing 10 ml of methanolic bile salt solution was weighed and the empty tube weight was subtracted to obtain the weight of the solution. The density of the solution was assessed as 0.84 mg/ml and volume was calculated.

5.7.3 ENZYMATIC ASSAY FOR BILE ACIDS

Semicarbazide HCl (0.5 M) was prepared in 0.02 M pyrophosphate buffer. NaOH was added to adjust the pH to 10.0. CAPS buffer (0.02 M) was prepared. A 20 ml volume of incubation reagent consisted of 1 ml of semicarbazide HCl phosphate buffer, 19 ml CAPS buffer, and 5 mg NAD.

Sample reaction tubes contained methanolic bile salt solution (1.0 ml) and incubation reagent (3.0 ml). Sample blank tubes were prepared with methanolic bile salt solution (1.0 ml), incubation reagent (3.0 ml), and distilled water (0.1 ml). Standard solutions of sodium taurocholate in 75:25 methanol:water (0.05 - 0.50 mMol) were prepared and aliquots (1.0 ml) of these standards were used in the standard reaction and standard blank tubes. All tubes were heated in a water bath at 37°C for 10 min. The 3-alpha-HSD enzyme solution

(0.1 ml of 3 units/ml) was then added to the standard and sample reaction tubes only, and all tubes were incubated at 37°C for 60 minutes. Absorbance of tubes was read at 340 nm against air. The difference in absorbance between reaction tubes and blank tubes (delta A) was analysed and sample bile acid content was determined from the standards.

6 SERUM ANALYSIS

Frozen serum samples were thawed at 4°C for 4 hours. A solution of EDTA (0.1 Mol), NaCl (0.15 Mol), and NaN_3 (0.2 Mol) was then added as 0.01 ml/ml serum to inhibit lipid peroxidation and bacterial activity. Serum was brought to 23°C before being aliquoted by volume. Total cholesterol, triglyceride, and lipoprotein cholesterol were analysed in serum obtained from each pig on the first and last day of both infusion periods.

6.1 TRIGLYCERIDE

The total triglyceride content of serum was analysed enzymatically with GPO-Trinder Reagent (Sigma Diagnostics, St. Louis, MO., procedure no. 339). Triglycerides are hydrolysed by lipoprotein lipase to glycerol and free fatty acids. The glycerol is phosphorylated to glycerol-1-phosphate by ATP in a reaction catalysed by glycerol kinase. Glycerol-

1-phosphate is then oxidized by glycerol phosphate oxidase to dihydroxyacetone phosphate and hydrogen peroxide. The H_2O_2 reacts with 4-aminoantipyrine and sodium N-ethyl-N-(3-sulfopropyl)m-anisidine (ESPA) in the presence of peroxidase to produce a quinoneimine dye, detected with an absorbance maximum at 540 nm. The quinoneimine dye molecule is formed stoichiometrically with the quantity of glycerol present.

A reagent mixture containing ATP (0.3 mMol), magnesium salt (3.0 mMol), 4-aminoantipyrene (0.15 mMol), ESPA (1.69 mMol), lipase (50000 U/litre), glycerol kinase (1000 U/litre), glycerol phosphate oxidase (2000 U/litre), peroxidase (2000 U/litre), and NaN_3 (0.5 g/litre) in a pH 7.0 buffer was used. Reagent mixture (1.0 ml) and distilled water (1.0 ml) were placed in each reaction tube. A 0.01 ml volume of either serum, glycerol standard (50 - 200 mg/dl), or distilled water (blank) was added to each reaction tube. Tubes were then agitated and incubated at 30°C for 10 minutes. Absorbance at 540 nm was determined and sample concentrations were calculated from standards.

Serum samples were analysed in triplicate. The quantity of glycerol present was analysed in each sample tube and converted to mg triglyceride/dl serum, expressed as triolein.

6.2 TOTAL CHOLESTEROL

The total cholesterol content of serum was analysed enzymatically with a Cholesterol Reagent preparation (Sigma Diagnostics, St. Louis, MO., procedure no. 352). Cholesterol esters are hydrolysed by cholesterol esterase to cholesterol and free fatty acids. Cholesterol is then oxidized by cholesterol oxidase to cholest-4-en-3-one and H_2O_2. The H_2O_2 reacts with 4-aminoantipyrine and p-hydroxybenzenesulfonate in the presence of peroxidase to produce a quinoneimine dye that is detected with an absorbance maximum at 500 nm. The quinoneimine dye molecule is formed stoichiometrically with the quantity of cholesterol present.

A reagent mixture containing 4-aminoantipyrine (0.3 mMol), p-hydroxybenzenesulfonate (30.0 mMol), cholesterol oxidase (300 U/litre), cholesterol esterase (100 U/litre), and peroxidase (1000 U/litre) in a pH 6.5 buffer was used. Reagent mixture (1.0 ml) and distilled water (1.0 ml) were placed in each reaction tube. A 0.01 ml volume of serum, cholesterol standard (50 - 300 mg/dl), or distilled water (blank) was added to each reaction tube. Tubes were then agitated and incubated at 37°C for 10 minutes. Absorbance at 500 nm was determined, and sample concentrations were determined from standards.

Serum samples were analysed in triplicate. The quantity of cholesterol present was determined in each sample tube and expressed as mg cholesterol/dl serum.

6.3 LIPOPROTEIN CHOLESTEROL

Lipoproteins were separated by density in serum using sequential floatation ultracentrifugation. The densities at which the major lipoproteins were isolated in pig serum were $1.006 \leq$ VLDL ≤ 1.03 g/ml, $1.03 \leq$ LDL ≤ 1.09 g/ml, and $1.09 \leq$ HDL ≤ 1.21 mg/dl. The procedure was developed based on methods used by Shuttler (PhD Thesis, 1989) with the following modifications. The total cholesterol content of each lipoprotein fraction was analysed enzymatically with a Cholesterol Reagent preparation (Sigma Diagnostics, St. Louis, MO., procedure no. 352).

Serum lipoprotein cholesterol was analysed in duplicate for each pig on the first and last day of both infusion periods. Cholesterol was expressed as mg lipoprotein cholesterol/dl serum.

6.3.1 VLDL

6.3.1.1 DENSITY ADJUSTMENT

Given the initial density of serum as 1.0063 g/ml at 22°C, precise quantities of KBr were added to 1.000 ml serum for density adjustment to 1.030 g/ml. At this density, the VLDL lipoprotein fraction of pig serum will float on top of the rest of the serum upon high speed centrifugation. The quantity of KBr needed to make this density adjustment was calculated using equation 11 (where v=1.000, d1=1.0063, d2=1.030, and p=0.2910). The mass of KBr required to adjust serum density was determined to be 0.0339 g KBr/ml of serum.

$$M = v(d2-d1)/(1-p*d2) \qquad (11)$$

where M = mass (g) of KBr to be added

d1 = original serum density (g/ml)

d2 = desired density (g/ml)

v = original serum volume (ml)

p = partial specific volume (ml/g) of KBr at 22°C and at a density of d2

KBr (Analytical Reagent Grade, 99.5%, BDH Chemicals, Toronto) was dried for 48 h at 85°C in a Precision Scientific convection oven, and stored in a dessicator at 22°C. A density adjusting solution of KBr in distilled water (0.1695

g/ml) was prepared. The density adjusting solution (0,200 ml), containing 0.0339 g KBr, was placed in 1 ml Beckman polycarbonate centrifuge tubes (11x34 mm, reorder no. 343778). These tubes were dried at 85°C for 12 hours to evaporate the water in the density adjusting solution, leaving only the KBr. Tubes were cooled to 22°C in a dessicator, and 1.000 ml serum was added to each tube. Tubes were agitated until all KBr was dissolved in the serum.

6.3.1.2 CENTRIFUGATION AND ISOLATION

Tubes were then centrifuged for 7 h at 12°C and 100,000 rpm on a Beckman TL-100 Ultracentrifuge, using a Beckman TLA 100.2 Fixed Angle Rotor (rmax = 38.9, rmin = 24.5). The floating VLDL layer appeared as a milky white layer and was drawn off carefully with a fine bore pipette under a concentrated incandescent light source. The VLDL layer was placed in a weighed 15 ml borosilicate glass disposable test tube. The pipette was rinsed with distilled water, and the washings were added to the tube containing VLDL. The tube volume was made up to about 10 ml with distilled water. The weight of the tube content was recorded and volume calculated assuming a density of 1 mg/ml. This was the diluted VLDL extract.

6.3.1.3 CHOLESTEROL ANALYSIS

Total cholesterol content of the VLDL fraction was analysed using the procedure described in 6.2 with the following modification. Diluted VLDL extract (0.7 ml), distilled water (1.0 ml), and reagent mixture (1.0 ml) were added to reaction tubes. Appropriate cholesterol standards and blanks were prepared. Tubes were then agitated and incubated at 37°C for 10 minutes. Absorbance at 500 nm was determined, and sample concentrations were calculated from the standards.

6.3.2 LDL

6.3.2.1 DENSITY ADJUSTMENT

Assuming the density of serum adjusted to 1.030 g/ml had changed negligibly upon removal of VLDL cholesterol, the volume of the remaining serum in the centrifuge tube after VLDL removal was made up to 1.000 ml with a KBr solution of density 1.030 g/ml. The composition of this volume adjusting solution was calculated to be 4.486 g KBr in 98.000 ml distilled water using equation 11 (where v=98.000, d1=0.998, d2=1.030, and p=0.2921).

Precise quantities of KBr were added to this 1.000 ml serum for density adjustment to 1.090 g/ml. At this density, the LDL lipoprotein fraction of pig serum floats on top of the rest of the serum upon high speed centrifugation. The quantity of KBr needed to make this density adjustment was calculated using equation 11 (where d1=1.030, d2=1.090, v=1.000, and p=0.2968). The mass of KBr required to adjust 1.000 ml serum of density 1.030 g/ml to 1.090 g/ml was determined to be 0.0890 g.

Dried KBr was used to make a density adjusting solution in distilled water of 0.445 g KBr/ml. Density adjusting solution (0.200 ml) containing 0.089 g KBr was added to 1 ml centrifuge tubes. These tubes were dried at 85°C for 12 hours to evaporate the water in the density adjusting solution, leaving only the KBr. Tubes were cooled to 22°C in a dessicator, and the 1.000 ml of serum (of density 1.030 g/ml) was added to each tube. Tubes were agitated until all KBr was dissolved in the serum.

6.3.2.2 CENTRIFUGATION AND ISOLATION

Tubes were centrifuged as described under 6.3.1.2. The floating LDL layer appeared as a slightly yellow layer and was drawn off and diluted as described under 6.3.1.2. This was the diluted LDL extract.

6.3.2.3 CHOLESTEROL ANALYSIS

Total cholesterol content of the LDL fraction was analysed using the procedure described under 6.2 with the following modification. Diluted LDL extract (0.15 ml), distilled water (1.0 ml), and reagent mixture (1.0 ml) were added to reaction tubes. Appropriate cholesterol standards and blanks were prepared. Tubes were then agitated and incubated at 37°C for 10 minutes. Absorbance at 500 nm was determined and sample concentrations calculated from standards.

6.3.3 HDL

6.3.3.1 DENSITY ADJUSTMENT

Assuming the density of serum adjusted to 1.090 g/ml had changed negligibly upon removal of LDL cholesterol, the volume of the remaining serum in the centrifuge tube after LDL removal was made up to 1.000 ml with a KBr solution of density 1.090 g/ml. The composition of this volume adjusting solution was calculated to be 13.240 g KBr in 97.000 ml distilled water using equation 11 (where v=97.000, d1=0.998, d2=1.090, and p=0.2990).

Precise quantities of KBr were added to this 1.000 ml serum of density 1.090 g/ml for density adjustment to 1.210 g/ml. At this density, the HDL lipoprotein fraction of pig serum floats on top of the rest of the serum upon high speed centrifugation. The quantity of KBr needed to make this density adjustment was calculated using equation 11 (where v=1.000, d1=1.090, d2=1.210, and p=0.3077). The mass of KBr required to adjust 1.000 ml serum of density 1.090 to 1.120 was determined to be 0.1913 g.

Dried KBr was used to make a density adjusting solution in distilled water of 0.445 g KBr/ml. The density adjusting solution (0.430 ml) containing 0.1913 g KBr, was added to 1 ml centrifuge tubes. These tubes were dried at 85°C for 12 hours to evaporate the water in the density adjusting solution, leaving only the KBr. Tubes were cooled to 22°C in a dessicator and the 1.000 ml of serum (of density 1.090 g/ml) was added to each tube. Tubes were agitated until all KBr was dissolved in the serum.

6.3.3.2 CENTRIFUGATION AND ISOLATION

Tubes were centrifuged for 14 hours under conditions described under 6.3.1.2. The floating HDL layer appeared as a slightly yellow layer and was drawn off and diluted as described under 6.3.1.2. This was the diluted HDL extract.

6.3.3.3 CHOLESTEROL ANALYSIS

Total cholesterol content of the HDL fraction was analysed using the procedure described under 6.2 with the following modification. Diluted HDL extract (0.3 ml), distilled water (1.0 ml), and reagent mixture (1.0 ml) were placed in reaction tubes. Appropriate cholesterol standards and blanks were prepared. Tubes were then agitated and incubated at 37°C for 10 minutes. Absorbance at 500 nm was determined and sample concentrations calculated from standards.

7 LIVER ANALYSIS

7.1 TOTAL CHOLESTEROL CONTENT

The total cholesterol content of each liver was analysed using a colorimetric method Test-Combination kit from Boehringer Mannheim (Cat. no. 139-050). To determine total cholesterol, a methylation procedure was used to free the esterified cholesterol.

Frozen liver samples were thawed at 4°C for 12 hours prior to analysis. Cholesterol content of the liver samples (1.0 - 3.0 g) taken from the last day of the second infusion period were analysed in triplicate. The procedure outlined

under 4.3.6 was used without further modification. Total liver cholesterol content was expressed as mg cholesterol/g liver, mg cholesterol/liver, and mg liver cholesterol/kg body weight.

8 STATISTICAL ANALYSIS

A SAS procedure for general linear models (proc GLM) employed the model shown in equation 12 for the two way factorial analysis of variance with interaction for most parameters measured. Proc GLM was used since an unequal number of observations can be accommodated for the different combinations of sources of variance.

$$Y_{ij} = w_i + x_j + w_i x_j + \text{error} \qquad (12)$$

where Y_{ij} = an individual observation

w_i = the effect of infusion period, where $_i$ has two levels

x_j = the effect of infusion treatment, where $_j$ has two levels

$w_i x_j$ = the effect of period and treatment interaction, where there are $_i x_j$ levels

If interaction ($w_i x_j$) effects were not significant in this model, the main effects model shown in equation 13 was used.

This model calculates variation due to individual animal differences.

$$Y_{ijk} = w_i + x_j + z_k + \text{error} \qquad (13)$$

where Y_{ijk} = an individual observation

w_i = the effect of infusion period, where $_i$ has two levels

x_j = the effect of infusion treatment where $_j$ has two levels

z_k = the effect of individual animals, where $_k$ has nine levels

If the main effect of infusion period (w_i) was not significant in either the two way factorial ANOVA or the main effects ANOVA, a paired T-test (SAS proc MEANS) was used to assess differences using each pig as its own control.

Liver analysis would not illustrate infusion period or animal effects, as livers were only extracted after the second infusion period. The four pigs that received propionate infusion were compared to the five pigs that received saline infusion in the second infusion period. Liver data were analysed in a one-way ANOVA shown in equation 14.

$$Y_j = x_j + \text{error} \qquad (14)$$

where Y_j = an individual observation

x_j = the effect of treatment,

where $_j$ has two levels

Proc CORR in SAS was used to calculate Pearson Correlation Coefficients (R) for correlations between serum lipid, ileal digesta and fecal sterol content, ileal minus fecal sterol difference, and liver parameters.

For all tests, the p value to accept a significant difference was set as a probability ≤ 0.05.

RESULTS

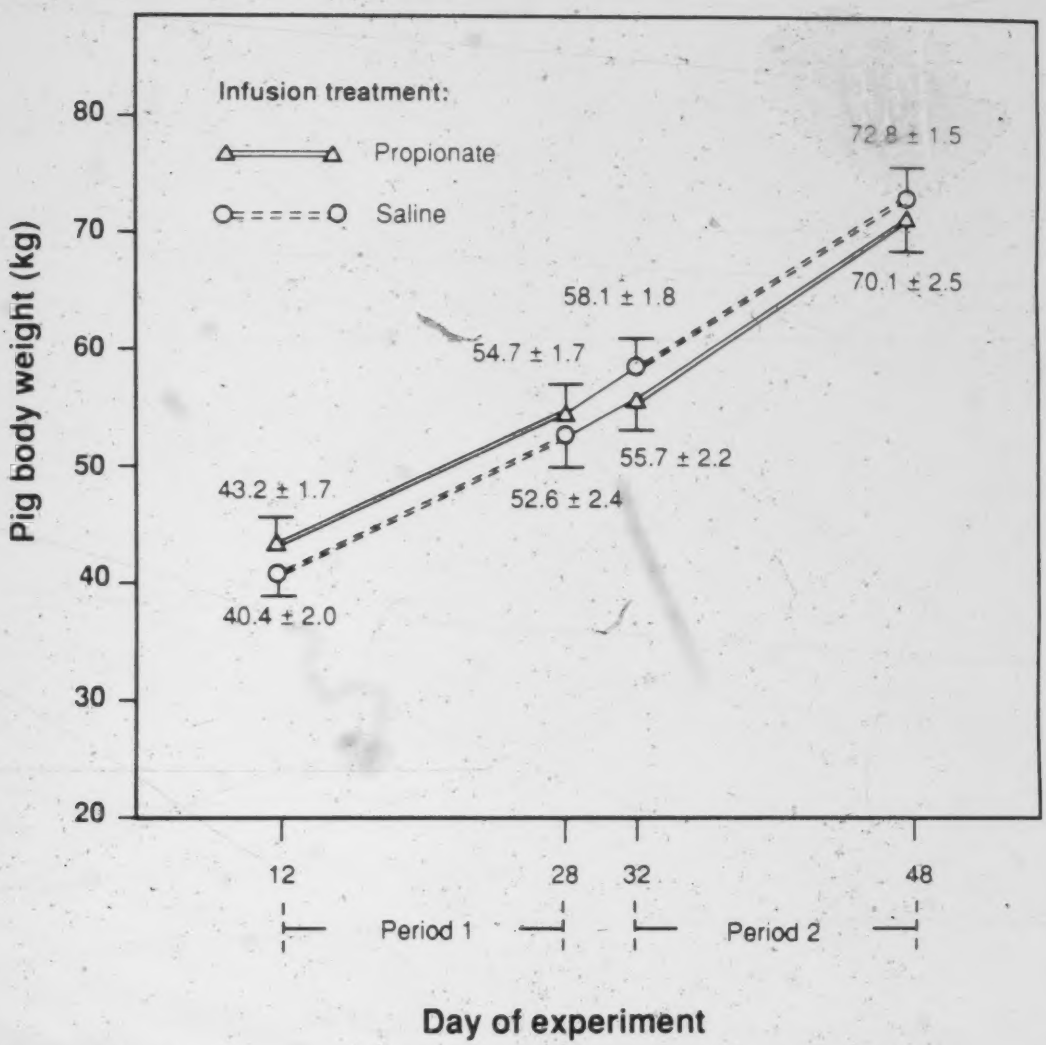
Pig weights (Figure 8) did not differ between treatment groups before or after either infusion period. Weight gain (Table 8) did not differ between the treatment groups, but was different between periods 1 and 2 ($p \leq 0.05$). Feed consumption was increased from 1800 g/day in period 1 to 2268 g/day in period 2 to maintain a constant feed intake of 109.3 g/kg$^{0.75}$ per day. This increase in consumption in the second period is reflected in the greater weight gain in the second period. Feed efficiency and percent body weight change (Table 8) were constant during both infusion periods and for both treatments.

Ileal digesta and fecal digestibility coefficients for nitrogen and organic matter are shown in Table 9. Nitrogen and organic matter digestibilities were not altered by infusion treatment. Fecal organic matter digestibility was greater in period 2 than in period 1 ($p \leq 0.05$).

Triglyceride, HDL cholesterol, LDL cholesterol, VLDL cholesterol, and total cholesterol content of serum collected after 16 days of infusion are presented in Table 10. Lipid content of serum collected on the first day of each infusion period did not differ between treatment groups or infusion

FIGURE 8

Pig weights[1]



[1] Values represent means ± SEM, n = 5, 4 for upper and lower lines, respectively.

TABLE 8

Weight gain, weight change, and feed efficiency[1]

	Infusion treatment	
	Propionate	Saline
Weight gain (kg)		
Period 1	11.5 ± 0.2 (5)a	11.2 ± 0.4 (4)a
Period 2	14.3 ± 0.8 (4)b	15.3 ± 0.8 (5)b
Average	12.8 ± 0.6 (9)	13.5 ± 0.9 (9)
Weight change (gain as % of start of period weight)		
Period 1	26.8 ± 1.3 (5)	28.5 ± 2.2 (4)
Period 2	25.9 ± 1.7 (4)	27.5 ± 2.5 (5)
Average	26.4 ± 1.0 (9)	27.9 ± 1.6 (9)
Feed efficiency (gain/consumption)		
Period 1	0.40 + 0.01 (5)	0.39 + 0.02 (4)
Period 2	0.39 + 0.02 (4)	0.42 + 0.02 (5)
Average	0.40 + 0.02 (9)	0.41 + 0.03 (9)

[1] Values are means ± SEM (n). Period values with different letters are significantly different $p \leq 0.05$.

TABLE 9

Ileal and fecal digestibility coefficients of nitrogen and organic matter[1]

	Infusion treatment	
	Propionate	Saline
	Digestibility coefficient (%)	
Ileal nitrogen		
Period 1	80.43 ± 2.31 (5)	72.83 ± 7.98 (4)
Period 2	79.25 ± 2.89 (4)	82.45 ± 0.87 (5)
Average	79.90 ± 1.71 (9)	78.17 ± 3.70 (9)
Ileal organic matter		
Period 1	76.96 ± 1.55 (5)	71.64 ± 7.08 (4)
Period 2	73.74 ± 4.44 (4)	77.90 ± 0.91 (5)
Average	75.53 ± 2.07 (9)	75.12 ± 3.13 (9)
Fecal nitrogen		
Period 1	84.41 ± 1.03 (5)	82.54 ± 0.97 (4)
Period 2	84.60 ± 1.03 (4)	84.30 ± 1.26 (5)
Average	84.50 ± 0.69 (9)	83.52 ± 0.83 (9)
Fecal organic matter		
Period 1	86.13 ± 0.45 (5)a	85.92 ± 0.28 (4)a
Period 2	88.95 ± 0.46 (4)b	87.82 ± 0.39 (5)b
Average	87.38 ± 0.58 (9)	86.98 ± 0.41 (9)

[1] Values are means ± SEM (n). Period values with different letters are significantly different, $p \leq 0.05$.

TABLE 10

Serum cholesterol content, triglyceride content, and LDL/HDL cholesterol ratio[1]

		Infusion treatment	
		Propionate	Saline
		mg/dl serum	
Total CH	Period 1	136.27 ± 4.71 (5)	112.91 ± 6.49 (4)
	Period 2	131.03 ± 8.32 (4)	119.73 ± 5.67 (5)
	Average	133.94 ± 4.30 (9)	116.70 ± 4.17 (9)*
HDL CH	Period 1	46.72 ± 2.06 (5)	39.90 ± 4.67 (4)
	Period 2	48.87 ± 3.37 (4)	43.75 ± 2.16 (5)
	Average	47.67 ± 1.70 (9)	42.04 ± 2.32 (9)
LDL CH	Period 1	74.57 ± 3.77 (5)	63.17 ± 2.14 (4)
	Period 2	70.52 ± 5.68 (4)	63.30 ± 2.89 (5)
	Average	72.77 ± 3.14 (9)	63.24 ± 1.76 (9)**
VLDL CH	Period 1	14.99 ± 1.63 (5)	9.82 ± 2.04 (4)
	Period 2	11.45 ± 1.77 (4)	12.68 ± 2.15 (5)
	Average	13.42 ± 1.28 (9)	11.41 ± 1.49 (9)
TG	Period 1	52.83 ± 3.69 (5)	38.12 ± 2.85 (4)
	Period 2	47.49 ± 4.98 (4)	47.45 ± 6.87 (5)
	Average	50.46 ± 2.97 (9)	43.30 ± 4.14 (9)
LDL CH/HDL CH			
	Period 1	2.28 ± 0.40 (5)	2.26 ± 0.87 (4)
	Period 2	1.97 ± 0.52 (4)	2.46 ± 0.63 (5)
	Average	2.14 ± 0.30 (9)	2.37 ± 0.38 (9)

[1]Values are means ± SEM (n).
*, ** - Average treatment values are significantly different, $p \leq 0.011$ and $p \leq 0.017$, respectively.
CH - cholesterol
TG - triglyceride

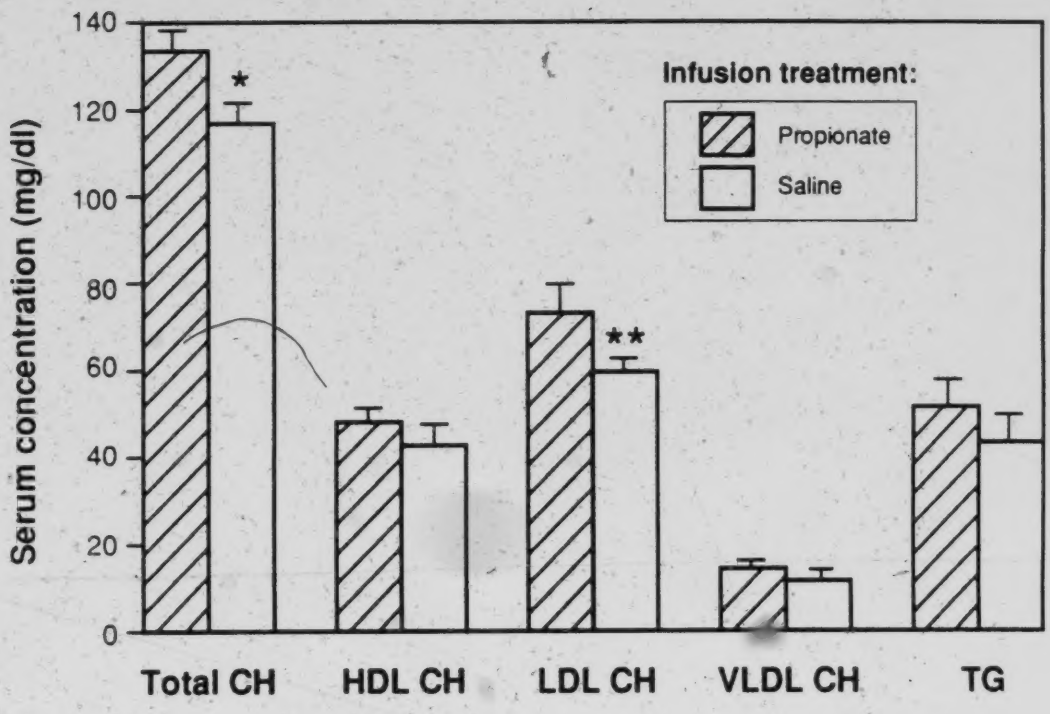
periods. These base-line values were (mean ± SEM mg/dl serum) 42.7 ± 2.7, 35.6 ± 2.5, 70.5 ± 3.3, 12.5 ± 1.2, and 118.8 ± 3.2 for triglyceride, HDL cholesterol, LDL cholesterol, VLDL cholesterol, and total cholesterol, respectively.

Total cholesterol and LDL cholesterol levels were different between treatments ($p \leq 0.011$, $p \leq 0.017$, respectively). Total cholesterol increased by 14.8%, from 116.7 mg/dl serum after saline infusion to 133.9 mg/dl after propionate infusion. LDL cholesterol increased by 15.1%, from 63.2 mg/dl after saline infusion to 72.8 mg/dl after propionate infusion. Other serum lipid categories, HDL cholesterol, VLDL cholesterol, and triglyceride, did not differ between infusion treatments, although serum lipids tended to increase with propionate infusion (Figure 9). The LDL cholesterol to HDL cholesterol ratio was not altered by infusion treatment.

Liver cholesterol content and liver weight measured at the end of the second infusion period are shown in Table 11. No differences in liver weight, total liver cholesterol, cholesterol concentration, liver weight expressed as a percentage of body weight, or liver cholesterol per kg of body weight were found between the treatment groups.

FIGURE 9

Effect of infusion treatment on serum lipids[1]



[1] Bars represent means ± SEM, n = 9
*,** - Lipid component means are significantly different, $p<0.011$, $p<0.017$ respectively

TABLE 11

Liver cholesterol and liver weight[1]

	Infusion treatment	
	Propionate (n=4)	Saline (n=5)
Liver weight (g)	1351.0 ± 35.7	1369.0 ± 31.9
Liver cholesterol (mg)	8876 ± 184	9137 ± 184
Liver cholesterol concentration (mg/g liver)	6.57 ± 0.10	6.78 ± 0.30
Liver weight as % of body weight	1.89 ± 0.03	1.92 ± 0.08
Liver cholesterol per kg body weight (mg/kg)	124.0 ± 2.0	127.9 ± 2.8

[1] Values are means ± SEM.

The bile acid and cholesterol content of the ileal digesta and the feces were not affected by infusion treatment (Table 12). Apparent colonic disappearance was calculated as the difference between ileal digesta sterol content and fecal sterol content as an estimate of the quantity of sterol either absorbed or degraded in the colon. Apparent colonic disappearance of sterols was not affected by treatment (Table 13).

Pearson Correlation Coefficients (R) for serum lipids are shown in Table 14. Total cholesterol correlated with HDL cholesterol ($p \leq 0.001$), LDL cholesterol ($p \leq 0.0001$), VLDL cholesterol ($p \leq 0.001$), and triglyceride ($p \leq 0.0002$). HDL cholesterol correlated with triglyceride ($p \leq 0.018$). LDL cholesterol correlated with VLDL cholesterol ($p \leq 0.04$), and triglyceride ($p \leq 0.016$). VLDL cholesterol correlated with triglyceride ($p \leq 0.001$).

Table 15 shows Pearson Correlation Coefficients for serum lipids with ileal digesta, fecal, and apparent colonic disappearance of sterol content. The only significant correlations that were found were negative relationships between VLDL cholesterol and ileal digesta cholesterol content ($p \leq 0.04$), and apparent colonic disappearance of cholesterol ($p \leq 0.03$).

TABLE 12

Sterol content of ileal digesta and feces[1]

		Infusion treatment	
		Propionate	Saline
		mmol/day	
Ileal			
BA	Period 1	4.55 ± 0.81 (5)	8.80 ± 1.93 (4)
	Period 2	9.78 ± 2.54 (4)	5.51 ± 0.49 (5)
	Average	6.88 ± 1.45 (9)	6.98 ± 1.01 (9)
CH	Period 1	2.20 ± 0.29 (5)	2.99 ± 0.24 (4)
	Period 2	3.29 ± 0.37 (4)	2.92 ± 0.11 (5)
	Average	2.67 ± 0.29 (9)	2.95 ± 0.11 (9)
BA+CH	Period 1	6.75 ± 0.64 (5)	11.79 ± 2.14 (4)
	Period 2	13.07 ± 2.90 (4)	8.43 ± 0.50 (5)
	Average	9.56 ± 1.66 (9)	9.92 ± 1.08 (9)
Fecal			
BA	Period 1	1.11 ± 0.26 (5)	1.64 ± 0.15 (4)
	Period 2	1.95 ± 0.25 (4)	1.05 ± 0.06 (5)
	Average	1.48 ± 0.23 (9)	1.31 ± 0.12 (9)
CH	Period 1	0.62 ± 0.07 (5)	0.74 ± 0.05 (4)
	Period 2	1.12 ± 0.04 (4)	1.15 ± 0.09 (5)
	Average	0.84 ± 0.10 (9)	0.97 ± 0.09 (9)
BA+CH	Period 1	1.73 ± 0.27 (5)	2.38 ± 0.11 (4)
	Period 2	3.07 ± 0.22 (4)	2.20 ± 0.08 (5)
	Average	2.32 ± 0.29 (9)	2.28 ± 0.07 (9)

[1] Values are means ± SEM (n).
BA - bile acid, CH - cholesterol

TABLE 13

Apparent colonic disappearance of sterols[1]

	Infusion treatment	
	Propionate	Saline
	mmol/day	
Bile acid		
Period 1	3.44 ± 0.75 (5)	7.16 ± 2.01 (4)
Period 2	7.83 ± 2.58 (4)	4.46 ± 0.50 (5)
Average	5.39 ± 1.36 (9)	5.66 ± 0.98 (9)
Cholesterol		
Period 1	1.58 ± 0.34 (5)	2.24 ± 0.21 (4)
Period 2	2.17 ± 0.37 (4)	1.77 ± 0.11 (5)
Average	1.84 ± 0.25 (9)	1.98 ± 0.13 (9)
Bile acid + cholesterol		
Period 1	5.03 ± 0.48 (5)	9.41 ± 2.17 (4)
Period 2	10.00 ± 2.93 (4)	6.23 ± 0.42 (5)
Average	7.24 ± 1.50 (9)	7.64 ± 1.07 (9)

[1] Values are means ± SEM (n).

TABLE 14

Pearson correlation coefficient matrix for serum lipids

R/ p>|R| under Ho: R=0 (n=18)

	VLDL CH	LDL CH	HDL CH	Total CH	TG	LDL CH/ HDL CH
VLDL CH	1.00	0.49	0.34	0.71	0.71	-0.10
	0.00	0.04	0.17	0.001	0.001	0.69
LDL CH		1.00	0.29	0.85	0.56	0.02
		0.00	0.24	0.0001	0.016	0.94
HDL CH			1.00	0.71	0.55	-0.14
			0.00	0.001	0.018	0.58
Total CH				1.00	0.76	-0.08
				0.00	0.0002	0.75
TG					1.00	-0.15
					0.00	0.55
LDL CH/HDL CH						1.00
						0.00

CH - cholesterol, TG - triglyceride

TABLE 15

Pearson correlation coefficient matrix for serum lipids, ileal and fecal sterol content, and apparent colonic disappearance

R/ p>|R| under Ho: R=0 (n=18)

	VLDL CH	LDL CH	HDL CH	Total CH	TG
Ileal					
BA	-0.14	0.25	0.02	0.12	0.02
	0.59	0.32	0.93	0.64	0.92
CH	-0.47	-0.17	0.06	-0.20	-0.05
	0.04	0.50	0.80	0.42	0.84
BA+CH	-0.19	0.20	0.03	0.07	0.01
	0.43	0.44	0.90	0.77	0.96
Fecal					
BA	-0.33	0.22	0.14	0.10	-0.09
	0.18	0.38	0.58	0.69	0.70
CH	-0.01	-0.08	0.02	-0.04	0.08
	0.98	0.73	0.94	0.87	0.74
BA+CH	-0.29	0.16	0.13	0.07	-0.05
	0.24	0.54	0.61	0.78	0.85
Apparent colonic disappearance					
BA	-0.09	0.23	0.01	0.11	0.04
	0.72	0.36	0.99	0.66	0.87
CH	-0.51	-0.15	0.06	-0.20	-0.10
	0.03	0.56	0.80	0.42	0.70
BA+CH	-0.16	0.18	0.01	0.07	0.02
	0.52	0.46	0.96	0.79	0.93

CH - cholesterol, TG - triglyceride, BA - bile acid

Positive correlations were found between fecal and ileal sterol content (Table 16). Ileal digesta bile acid content was correlated with ileal digesta cholesterol content ($p \leq 0.004$), ileal digesta bile acid + cholesterol content ($p \leq 0.0001$), and fecal bile acid + cholesterol content ($p \leq 0.04$). Ileal digesta cholesterol content correlated with ileal digesta bile acid + cholesterol content ($p \leq 0.0006$), and fecal bile acid + cholesterol content ($p \leq 0.02$). Ileal digesta bile acid + cholesterol correlated with fecal bile acid + cholesterol content ($p \leq 0.03$). Fecal bile acid and cholesterol correlated with fecal bile acid + cholesterol ($p \leq 0.0001$ and $p \leq 0.05$, respectively).

Positive correlations were found between all apparent colonic disappearance of sterol categories (Table 17). Ileal digesta contents of bile acid, cholesterol, and bile acid + cholesterol were correlated with apparent colonic disappearance of bile acid, cholesterol, and bile acid + cholesterol. However, significant correlations were not found between the content of any fecal sterols and apparent colonic disappearance of any sterol categories (Table 17).

Table 18 shows correlation coefficients for liver weight and liver cholesterol values and serum lipids. Liver cholesterol concentration was correlated positively with liver weight ($p \leq 0.016$). Both total liver cholesterol and liver

TABLE 16

Pearson correlation coefficient matrix for fecal and ileal sterol content

R/ p>|R| under Ho: R=0 (n=18)

	Ileal			Fecal		
Ileal	BA	CH	BA+CH	BA	CH	BA+CH
BA	1.00	0.64	0.99	0.39	0.31	0.49
	0.00	0.004	0.0001	0.11	0.20	0.04
CH		1.00	0.73	0.41	0.40	0.54
		0.00	0.0006	0.09	0.10	0.02
BA+CH			1.00	0.41	0.34	0.52
			0.00	0.09	0.16	0.03
Fecal						
BA				1.00	0.02	0.89
				0.00	0.94	0.0001
CH					1.00	0.47
					0.00	0.05
BA+CH						1.00
						0.00

BA - bile acid, CH - cholesterol

TABLE 17

Pearson correlation coefficient matrix for ileal, fecal, and apparent colonic disappearance of sterols

R/ p>|R| under Ho: R=0 (n=18)

	Apparent colonic difference		
Ileal	BA	CH	BA + CH
BA	0.99 0.0001	0.55 0.02	0.99 0.0001
CH	0.61 0.007	0.90 0.0001	0.70 0.001
BA + CH	0.98 0.001	0.63 0.005	0.99 0.001
Fecal			
BA	0.25 0.31	0.43 0.07	0.30 0.23
CH	0.33 0.18	-0.04 0.88	0.29 0.24
BA + CH	0.37 0.13	0.36 0.14	0.39 0.10
Apparent colonic disappearance			
BA	1.00 0.00	0.51 0.03	0.99 0.0001
CH		1.00 0.00	0.62 0.006
BA + CH			1.00 0.00

BA - bile acid, CH - cholesterol

TABLE 18

Pearson correlation coefficient matrix for liver weight, liver cholesterol and serum lipids

R/ p>|R| under Ho: R=0 (n=9)

	LIVWT	CH/gL	CH/L	%L/BW	CH/kgBW
LIVWT	1.00 0.00	-0.76 0.016	0.06 0.87	0.41 0.28	-0.51 0.16
CH/gL		1.00 0.00	0.53 0.14	-0.65 0.05	0.46 0.21
CH/L			1.00 0.00	-0.69 0.04	-0.12 0.76
%L/BW				1.00 0.00	0.34 0.37
CH/kgBW					1.00 0.00
VLDL CH	0.09 0.82	-0.28 0.47	-0.43 0.25	0.69 0.038	0.49 0.18
LDL CH	0.59 0.09	-0.46 0.21	0.08 0.83	0.19 0.63	-0.32 0.41
HDL CH	-0.30 0.48	0.06 0.88	-0.32 0.41	0.19 0.62	0.32 0.40
Total CH	0.27 0.48	-0.34 0.37	-0.20 0.60	0.38 0.31	0.07 0.86
TG	0.41 0.28	-0.45 0.23	-0.29 0.45	0.74 0.02	0.34 0.37

CH - cholesterol, TG - triglyceride,
LIVWT - liver weight, CH/gL - cholesterol/g liver,
CH/L - total liver cholesterol,
%L/BW - liver weight as % body weight,
CH/kgBW - liver cholesterol/kg body weight.

cholesterol concentration were negatively correlated with liver weight expressed as a percentage of body weight ($p \leq 0.04$, $p \leq 0.05$, respectively). Liver weight expressed as a percentage of body weight was positively correlated with VLDL cholesterol and triglyceride ($p \leq 0.038$, $p \leq 0.02$, respectively). Liver parameters were not correlated with ileal digesta and fecal sterol contents (Table 19).

TABLE 19

Pearson correlation coefficient matrix for liver weight and cholesterol with ileal content, fecal content, and apparent colonic disappearance of sterols

R/ p>|R| under Ho: R=0 (n=9)

	LIVWT	CH/gL	CH/L	%L/BW	CH/kgBW
Ileal					
BA	0.15 0.69	-0.34 0.37	-0.32 0.40	0.19 0.63	-0.18 0.65
CH	0.11 0.78	-0.10 0.79	-0.03 0.93	-0.08 0.85	-0.21 0.59
BA+CH	0.15 0.70	-0.31 0.41	-0.29 0.41	0.16 0.69	-0.20 0.60
Fecal					
BA	0.14 0.72	-0.17 0.66	-0.04 0.92	-0.17 0.67	-0.42 0.26
CH	-0.16 0.68	0.14 0.71	0.15 0.70	0.16 0.68	0.54 0.13
BA+CH	0.10 0.80	-0.14 0.72	0.00 1.00	-0.13 0.73	-0.30 0.44
Apparent colonic disappearance					
BA	0.14 0.72	-0.33 0.38	-0.33 0.38	0.22 0.56	-0.14 0.72
CH	0.15 0.70	-0.15 0.71	-0.08 0.84	-0.12 0.76	-0.39 0.31
BA+CH	0.14 0.71	-0.31 0.41	-0.31 0.42	0.18 0.64	-0.17 0.66

CH - cholesterol, BA - bile acid,
LIVWT - liver weight, CH/gL - cholesterol/g liver,
CH/L - total liver cholesterol,
%L/BW - liver weight as % body weight,
CH/kgBW - liver cholesterol/kg body weight.

DISCUSSION

Although propionate infusion provided 3.5% extra kcals, the increase in energy was not accompanied by an increase in weight gain or % weight change during propionate infusion (Table 8). Weight gain was significantly different between the first and second infusion periods ($p \leq 0.05$, Table 8). The increase in gain during the second period was attributable to increased consumption. Pig body weight at the start of the second infusion period was 36% greater than at the start of the first infusion period (Figure 8). Metabolic body weight ($kg^{0.75}$) was increased by 26%. Feed intake was purposefully increased to maintain a constant $kcal/kg^{0.75}$ ratio, thus providing a constant excess of energy in both periods. It has been shown that cholesterol synthesis is decreased when a mild kcal restriction is imposed (Grundy, 1978). Due to this feed consumption correction for metabolic body weight, % body weight change and feed efficiency did not differ between infusion periods (Table 8). There was no significant period effect in serum lipid analysis (Table 10), indicating this correction was justified.

Digestibilities of dietary components were assessed to ensure that any differences in serum lipids were not attributable to altered absorption of nutrients. Fich et al (1989) illustrated stimulation of ileal emptying by bursts of

phasic contractions upon bolus infusion of SCFAs to the ileum compared to saline infusion in both fasting and post-prandial dogs. Bolus SCFA infusion through ileal intubation of humans showed enhanced motility relative to saline and air infusion in humans (Kamath et al, 1988). This effect could lead to nutrient malabsorption in the small intestine. Propionate provision may enhance gut integrity, which could enhance nutrient absorption.

Fecal digestibilities did not differ between infusion treatments (Table 9). However, there was an increase in fecal organic matter digestibility in the second period (Table 9). The significance of this relatively small increase of about 2% was due in part to very little variation among animals in each period. This increase in digestibility was not reflected in a concurrent increase in feed efficiency (Table 8). In infusion period 1, one animal in the saline infusion group had lower ileal nitrogen and organic matter digestibility than the others (Table 9), making the mean of the four animals seem lower than would be expected. This animal was not excluded, as no differences were detected between treatment groups. Differences in serum lipids are not attributable to nutrient losses through excretion.

The results of the serum lipid analyses (Table 10) do not support the hypothesis that propionate infusion lowers serum

lipids, but instead suggest that propionate infusion increases serum cholesterol, mainly in the LDL cholesterol fraction. Although serum was stored at -30°C for 2 to 3 months prior to analysis, it is unlikely that the storage period affected lipoprotein cholesterol or triglyceride concentrations. Changes in human lipoprotein cholesterol or triglyceride fractions have not been found between fresh serum and serum stored at -20°C for 27 weeks prior to separated by density gradient ultracentrifugation (Tiedink and Katan, 1988).

Pigs were fed a high fat diet as a model of the hyperlipidemic human. The range of serum triglyceride, cholesterol and lipoprotein cholesterol concentrations found in the present study (Table 10) were in agreement with other studies that have shown increased tallow consumption to cause hyperlipidemia in pigs (Boila et al, 1981; Thacker and Bowland, 1981). A hyperlipidemic pig model was used because fiber supplementation for the purpose of lowering serum cholesterol is of more practical concern for those individuals with high serum cholesterol.

In the present study, excess cholesterol was not supplemented in the diet beyond the quantity found in the tallow added to the diet. Inclusion of a larger quantity may have masked any effects that propionate infusion had on hepatic cholesterol synthesis. Pigs fed diets supplemented

with 1% cholesterol and 0.5% cholic acid, with 14% fat (w/w) had serum cholesterol levels of about 400% of normal pigs consuming a grower diet (Ahrens, 1986). At this dramatically increased level, results would be difficult to interpret, and would not be representative of the hypercholesterolemic human.

Normal pig serum cholesterol ranges around 80 mg/dl (2 mmol/litre) (Mahley, 1974). In the present study, pigs consuming the high fat diet had serum cholesterol values ranging about 150% of normal (Table 10) and were considered a representative model of humans with elevated serum cholesterol levels.

Propionate feeding at a level of 6 and 9% (w/w) of an ad libitum fed diet resulted in a decreased diet consumption, compared to the 0% control and the 3% propionate supplemented diet (Thacker and Bowland, 1981). Decreased caloric intake may be responsible for the observed decrease in serum cholesterol at 6 and 9% dietary propionate supplementation levels (Thacker and Bowland, 1981). No differences in serum cholesterol were observed in pigs fed the 3% (w/w) propionate supplemented diet. The quantity of sodium propionate infused in the present study was equal to 2.5% of the diet (w/w) or 3.5% of the kcals.

Sodium propionate feeding in rats as 5% (w/w) of the diet also showed low diet compliance, resulting in a 20-25% lower consumption during the first two days of a ten day feeding period (Illman et al, 1988). A decrease in total plasma cholesterol resulted in these rats after the ten days of feeding, as compared to control rats. Hepatic cholesterol synthesis was measured through tritiated water incorporation from intraperitoneal injection one hour prior to liver extraction. Liver cholesterol concentration was increased upon propionate feeding, but synthesis was unchanged. Provision of 7.5 g sodium propionate/day (1.5% of daily kcal) for seven weeks increased triglyceride and HDL cholesterol in humans (Venter et al, 1990).

Liver cholesterol content and liver weight were evaluated at the end of the second infusion period only. The four pigs receiving propionate infusion were compared to the five pigs receiving saline infusion. Individual variation between animals could not be accounted for, and considerable variation existed within a treatment group. Differences in liver cholesterol were not detected between treatments (Table 11).

Sterol content of ileal digesta and feces did not show differences between treatments (Table 12). Correlations between serum lipids and ileal digesta or fecal sterol content were not significant (Table 15). Differences in serum lipids

were not due to alterations in sterol absorption or excretion. Bacterial degradation of bile acids in the human colon can occur so that degradation products are unrecognizable to the enzyme 3-alpha-hydroxysteroid dehydrogenase (3-alpha-HSD) (Lepage and Roy, 1988). An estimated 15% of bile acids, present as sulfated or keto bile acids are also undetected by 3-alpha-HSD (Roy and Weber, 1977). As fecal bile acid losses can be used as a rough estimate of bile acid synthesis (Hofmann, 1977; Lepage and Roy, 1988), it is unlikely that differences in bile acid synthesis occurred between treatment groups.

Apparent colonic disappearance of sterols indicate the quantity of sterol either reabsorbed between the distal ileum and the rectum, or those altered or degraded to a form unrecognizable to 3-alpha-HSD. The lack of difference between treatment groups (Table 13) indicates enhanced reabsorption of sterol is not responsible for the observed increase in serum cholesterol.

The significant correlations found between total cholesterol, with triglyceride and all serum lipoprotein cholesterol fractions (Table 14) emphasizes the tendency for all lipoprotein cholesterol concentrations and triglyceride to increase upon propionate infusion. However, this tendency

was not statistically significant in the analysis of variance (Table 10).

Ileal and fecal sterol content did not correlate significantly with serum lipids, except for ileal digesta cholesterol which correlated negatively with VLDL cholesterol ($p \leq 0.04$, Table 15). VLDL is synthesized in both liver (Marsh and Whereat, 1959), and intestinal mucosa (Gangl and Ockner, 1975). The correlation implies that increased upper gastrointestinal cholesterol absorption, resulting in less cholesterol entering the colon, increases the quantity of cholesterol incorporated into VLDL in mucosal VLDL synthesis. VLDL cholesterol also correlated negatively with apparent colonic disappearance of cholesterol (Table 15), suggesting an increase in colonic cholesterol absorption also results in increased incorporation of cholesterol into VLDL. No other apparent colonic disappearances of sterols were correlated to serum lipids (Table 15). As triglyceride is mainly carried by VLDL in serum, the lack of correlation of ileal digesta cholesterol or apparent colonic disappearance of cholesterol with triglyceride suggests VLDL composition is altered by absorption of cholesterol from the intestine (Table 15).

Ileal digesta bile acid was correlated with cholesterol content ($p \leq 0.004$, Table 16). This implies that enhanced upper

gastrointestinal sterol absorption proceeds through a mechanism that increases absorption of both sterol classes.

Fecal bile acid and cholesterol content correlated with both ileal digesta bile acid and cholesterol content ($p \leq 0.04$ and $p \leq 0.02$, respectively, Table 16) indicating that a higher quantity of sterol entering the colon is accompanied by increased sterol excretion, although increased apparent colonic disappearance of sterol is also observed when a higher quantity of sterol enters the colon. This is evidenced by correlations found between apparent colonic disappearance of both bile acids and cholesterol with digesta bile acid and cholesterol content (Table 17).

Correlations of liver weight as percentage of body weight with VLDL cholesterol ($p \leq 0.04$) and triglyceride ($p \leq 0.02$) were found (Table 18). This suggests that a larger liver weight was associated with higher serum triglyceride and VLDL cholesterol. Total cholesterol content of liver and cholesterol concentration in liver were negatively correlated with liver weight ($p \leq 0.05$ and $p \leq 0.04$, respectively, Table 18). This suggests that a larger liver weight was not associated with increased liver cholesterol.

No correlations were significant between ileal and fecal sterols or apparent colonic disappearance of sterols with the

liver parameters assessed (Table 19). This suggests that no relationship exists between sterol reabsorption or excretion and liver cholesterol.

The increase in serum total cholesterol and LDL cholesterol cannot be attributed to enhanced sterol conservation. The occurrence of either mobilization of tissue cholesterol to serum, enhanced cholesterol synthesis, or down-regulation of serum LDL clearance are alternate explanations for the observed serum cholesterol increase. In the present study, LDL cholesterol was the main serum cholesterol fraction to increase (Table 10). LDL cholesterol increases in serum have been highly correlated with an increased incidence of atherosclerosis in other studies (Wilkenson, 1948; Epstein et al, 1959; Slack, 1969). The origin of the cholesterol found in atherosclerotic lesions is from LDL cholesterol (Newman and Zilversmit, 1962; Jagannathan et al, 1974). Thus, elevated LDL cholesterol is generally associated with tissue cholesterol deposition rather than mobilization.

Increases in serum cholesterol upon ingestion of saturated fats is well documented (Hegsted et al, 1965; McNamara et al, 1987; Keys et al 1957). Studies in humans show increases in serum cholesterol induced by saturated fat feeding are largely in the LDL fraction (Vega et al, 1982; Mattson and Grundy, 1985; Grundy, 1986). This response also

occurred upon propionate infusion in the present study. Enhanced cholesterol synthesis or decreased serum LDL cholesterol clearance due to propionate are likely explanations for the increase in serum cholesterol.

Isolated rat hepatocytes incubated in 15-30 mMol propionate showed inhibited cholesterol synthesis (Anderson and Bridges, 1981). Bovine liver slices incubated in 15 mMol propionate resulted in inhibition HMG-CoA synthase activity (Bush and Milligan, 1971), and 18 mMol propionate inhibited cholesterol synthesis in perfused rat liver, although 1 mMol propionate had no effect (Illman et al, 1988). Hepatic portal blood concentration of propionate in non-fasted ad libitum-fed rats was 0.22 mMol, and total SCFA concentration was 1.6 mMol (Illman et al, 1988). In vitro incubation at extraphysiological levels (15 and 18 mMol) are difficult to interpret and may not be representative of in vivo hepatic cholesterol synthesis. Diets containing 5% sodium propionate fed to rats for 10 days resulted in increased liver cholesterol content although no change in cholesterol synthesis was observed and total serum cholesterol decreased (Illman et al, 1988).

To address this problem further, it would be beneficial to assess tissue cholesterol from biopsies done while animals experience different treatments to remove effects of

individual variability. A liver biopsy for assessment of hepatic cholesterol synthesis while an animal experiences different treatments, or a drug interfering with cholesterol synthesis such as AY-9944 (Boila et al, 1981), could be used to see if synthesis or clearance of cholesterol is responsible for serum cholesterol alterations.

To ensure animals receive the same quantity of energy during both treatments, extra energy in the form of carbohydrate could be given to animals during the saline infusion treatment to compensate for the quantity of energy provided from propionate infusion. This may address the question of whether the increase in serum cholesterol is due to excess energy, or the form the energy is provided in. Also, a slight energy restriction would ensure propionate is used for energy, and is not incorporated into fatty acids for storage. Infusing ^{14}C labelled propionate would show the fate of the infused molecules and the degree of incorporation in the tissues or in serum cholesterol.

Pigs have a longer GI retention time than humans, and fermentation can occur in the cecum. Pigs metabolize considerably more SCFAs than humans (Van Soest et al, 1982). Pigs do not consume as much saturated fat and cholesterol as

humans, due to a grain based diet. These results must be interpreted for humans with species differences in mind, as outlined under 7.2.

Although cecal propionate infusion may alter hepatic cholesterol synthesis or serum cholesterol clearance, its metabolism does not appear to be the main mechanism of the observed serum cholesterol lowering effect upon fermentable fiber supplementation. The observations made in this study reflect the conflicting reports of the role of propionate on serum cholesterol regulation.

REFERENCES

Ahrens F, Hagemeister H, Pfeuffer M, Barth CA. Effect of oral and intracaecal pectin administration on blood lipids in minipigs. J Nutr 1986;116:70-76.

American Diabetic Association. Position of the American Dietetic Association: health implications of dietary fiber. J Am Dietetic Assn 1988;88:216-221.

American Heart Association. Nutrition Committee and the council on Arteriosclerosis, AHA. Recommendations for treatment of hyper-lipidemia in adults. 1984;69:1067A

Anderson JW, Chen W-JL. Plant fiber: carbohydrate and lipid metabolism. Am J Clin Nutr 1979;32:346-363.

Anderson JW, Bridges SR. Plant fibre metabolites alter hepatic glucose and lipid metabolism. Diabetes 1981; 30(1S):133A.

Anderson JW, Story L, Sieling B, Chen W-JL. Hypocholesterolemic effects of high-fibre diets rich in water-soluble plant fibres: long-term studies with oat-bran and bean-supplemented diets for hypercholesterolemic men. J Can Diet Assoc 1984(a); 45:140-149.

Anderson JW, Story L, Sieling B, Chen W-JL, Petro MS, Story G. Hypocholesterolemic effects of oat-bran or bean intake for hypercholesterolemic men. Am J Clin Nutr 1984(b);40:1146-1155.

Anderson JW. Cholesterol-lowering effects of canned beans for hypercholesterolemic men. Clin Res 1985; 33: 871A.

Anderson JW. Dietary fiber in nutrition management of diabetes. In: Vahouny GV, Kritchevsky D, eds. Dietary fiber in health and disease. New York: Plenum, 1986:343-360.

Anderson JW, Gustafson NJ. Hypocholesterolemic effects of oat and bean products. Am J Clin Nutr 1988;48:749-753.

AOAC Official Methods of Analysis. Williams S (ed.) 14th Edition. AOAC, Inc., Arlington, VA. 1984; 153-157.

Argenzio RA, Southworth M. Sites of organic acid production and absorption in gastrointestinal tract of the pigs. Am J Physiol 1974;228:454-460.

Argenzio RA, Southworth M, Lowe JE, Stevens CE. Interrelationship of Na. HCO3, and volatile fatty acid transport by equine large intestine. Am J Physiol 1977;233:E469-478.

Arvanitakis C, Stammes CL, Folscroft J, Beyer P. Failure of bran to alter diet-induced hyperlipidemia in the rat. Proc Soc Exp Biol Med 1977;154:550-552.

Baekey PA, Cerda JJ, Burgin CW, Robbins FL, Baumgartner TG. Effect of grapefruit pectin on plasma cholesterol and development of atherosclerosis in miniature swine. J Parent Ent Nutr 1987;11:(s1)8 abs 36.

Balmer J, Zilversmit DB. Effect of dietary roughage on cholesterol absorption, cholesterol turnover and steroid excretion in the rat. J Nutr 1974; 104:1319-1328.

Bergman EN. In: McDonald IW, Warner ACI, eds. Digestion and Metabolism in the Ruminant, University of New England Publishing Unit, Austrailia 1975:292-305.

Bingham S. Definitions and intakes of dietary fibre. Am J Clin Nutr 1987;45:1226-1231.

Bjornhag G, Jonsson E. Replaceable gastrointestinal cannulas for small ruminants and pigs. Livestock Prod Sci 1984;11:179-184.

Boila J, Salomons MO, Milligan LP, Aherne FX. The effect of dietary propionic acid on cholesterol synthesis in swine. Nutr Rep Intl 1981;23:1113-1121.

Bond JH, Engel RR, Levitt MD. Factors influencing pulmonary methane excretion in man. J Exp Med 1971;133:572-588.

Bosaeus IG, Andersson HB. Short-term effect of two cholesterol-lowering diets on sterol excretion in ileostomy patients. Am J Clin Nutr 1987;45:54-59.

Bosaeus I, Carlsson NG, Sandberg AS, Andersson H. Effect of wheat bran and pectin on bile and cholesterol excretion in ileostomy patients. Hum Nutr Clin Nutr 1986; 40C:429-440.

Bougat M. Occurrence, absorption and metabolism of short chain fatty acids in the digestive tract of mammals. Comp Biochem Physiol 1987;86B;439-472.

Brieger L. Ueber die fluchtigen bestandtheile der menschlicher excremente. J Prakt Chemie (Neue Folge) 1978;17:124-138.

Burkitt DP, Trowell HC. Refined carbohydrate foods and disease: the implications of dietary fibre. London: Academic Press 1975.

Bush RS, Milligan LP. Study of the mechanism of inhibition of ketogenesis by propionate in bovine liver. Can J Anim Sci 1971;51:121-127.

Calvert RJ, Kritchevsky ES, Einhorn E, Klurfeld DM, Kritchevsky D. An improved method for oxidation of chromium(III)oxide-containing fecal samples by using sodium peroxide fusion. Am J Clin Nutr 1989; 49:901-903.

Chapman MJ. Animal lipoproteins: chemistry, structure and comparative aspects. J Lipid Res 1980;21:789-853.

Chen W-JL, Anderson JW, Jennings D. Propionate may mediate the hypercholesterolaemic effects of certain soluble plant fibres in cholesterol-fed rats. Proc Soc Exptl Biol Med 1984;75:215-218.

CRC Handbook of Chemistry and Physics. West RC (ed.) 65 th Edition. CRC Press Inc., Boca Raton, Florida, 1984.

Cummings JH, Branch W, Jenkins DJ, Southgate DJA, Houston H, James WPT. Colonic response to dietary fibre from carrot, cabbage, apple, bran and guar gum. Lancet 1978;1:5-9.

Cummings JH, Southgate DA, Branch WJ, Wiggins HS, Houston H, Jenkins DJA, Jivraj T, Hill MJ. The digestion of pectin in the human gut and its effect on calcium absorption and large bowel function. Br J Nutr 1979;41:477-485.

Cummings JH. Short chain fatty acids in the human colon. Gut 1981;22:763-779.

Cummings JH. Consequences of the metabolism of fibre in the human large intestine. In: Vahouny GV, Kritchevsky D, eds. Dietary fiber in health and disease. New York: Plenum, 1982:9-22.

Dankert J, Zijlstra JB, Wolthers BF. Volatile fatty acids in human peripheral and portal blood: quantitative determination by vacuum distillation and gas chromatography. Clin Chim Acta 1981;110:301-307.

deLange CF, Sauer WC, Mosenthin R, Souffrant WB. The effect of feeding different protein-free diets on the recovery and amino acid composition of endogenous protein collected from the distal ileum and feces in pigs. J Anim Sci 1989;67:746-754.

deLange CF, Sauer WC, Souffrant WB. The effect of protein status of the pig on the recovery and amino acid composition of endogenous protein in digesta collected from the distal ileum. J Anim Sci 1989;67:755-762.

Durrington PN, Manning AP, Bolton CH, Hartog M. Effect of pectin on serum lipids and lipoproteins, whole-gut transit-time, and stool weight. Lancet 1976; 2:394-396.

Epstein FH, Block WD, Hand EA, Francis T. Familial hypercholesterolemia, xanthomatosis and coronary heart disease. Amer J Med 1959;26:39.

Fidge N, Smith GD. Isolation and characterization of pig lipoprotein subfractions within the density range 1.006-1.090 g/ml. Artery 1975;1:406-427.

Fich A, Phillips SF, Hakim NS, Brown ML, Zinsmeister AR. Stimulation of ileal emptying by short-chain fatty acids. Dig Dis Sci 1989;34:1516-1520.

Fleming SE, Acre D. Using the pig to study fermentation in the gut. Swine in Biomedical Research, Vol I (ME Tumbleson) Plenum Press, NY. 1986:123-134.

Fleming SE, Wasilewski MM. Using the pig as a tool for studying fermentation in the human gut. Nutr Rep Int 1984;30:825-834.

Food and Drug Administration. Physiological Effects and Health Consequences of Dietary Fiber. Pilch SM (ed.) Life Sciences Research Office, Federation on American Societies for Experimental Biology, Bethseda, Maryland 1987:159-163.

Friend DW, Nicholson JWG, Cunningham HM. Volatile fatty acid and lactic acid content of pig blood. Can J Anim Sci 1964;44:303-308.

Gallaher D, Schneeman BO. Intestinal interaction of bile acids, phospholipids, dietary fibers, and cholestyramine. Am J Physiol 1986;250:G420-G426.

Gangl A, Ockner RK. Intestinal metabolism of lipids and lipoproteins. Gastroent 1975;68:167-186.

Garcia-Lopez JS, Lee K. Iron binding by fiber is influenced by competing minerals. J Food Sci 1985;50:424-428.

Graham H, Aman P. The pig as a model in dietary fibre digestion studies. Scand J gastroent 1987; 22(129S):55-61.

Graham H, Aman P, Newman RK, Newman CW. Use of a nylon-bag technique for pig feed digestibillity studies. Br J Nutr 1985;54:719-726.

Grimble G. Fibre, fermentation, flora and flatus. Gut 1989;30:6-13.

Grundy SM. Cholesterol metabolism in man. West J Med 1978;128:13-25.

Grundy SM. Comparison of monounsaturated fatty acids and carbohydrates for lowering plasma cholesterol. N Eng J Med 1986;314:745-748.

Havel RJ, Eder HA, Bragdon JH. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. J Clin Invest 1955; 34:1345-1353.

Health and Welfare Canada. Report of the Expert Advisory Committee on Dietary Fibre. Ottawa, Canada. Health Protection Branch. October 1985.

Hegsted DM, McGandy RB, Myers ML, Stare FJ. Quantitative effects of dietary fat on serum cholesterol in man. Amer J Clin Nutr 1965:17:281-295.

Hillman LC, Peters SG, Fisher CA, Pomare EW. Effect of the fibre components pectin, cellulose, and lignin on bile salt metabolism and biliary lipid composition in man. Gut 1986;27:29-36.

Hofmann AF. The enterohepatic circulation of bile acids in man. Clin Gastroent 1977;6:3-24.

Hogben CAM, Tocco DJ, Brodie BB, Schanker LS. On the mechanism of intestinal absorption of drugs. J Pharmacol Exp Ther 1959;125:275-282.

Holdeman LV, Good IJ, Moore WE. Human fecal flora: variation in bacterial composition within individuals and a possible effect of emotional stress. Appl Environ Microbiol 1976;31:359-375.

Hoverstad T. Studies of short chain fatty acid absorption in man. Scand J Gastroent 1986;21:257-260.

Illman RJ, Topping DL, McIntosh GH, Trimble RP, Storer GB, Taylor MN, Cheng B. Hypocholesterolaemic effects of dietary propionate: studies in whole animals and perfused rat liver. Ann Nutr Met 1988;32:97-107.

Jagannathan SN, Connor WE, Baker WH, Bhattacharyya AK. The turnover of cholesterol in human atherosclerotic arteries. J Clin Invest 1974;54:366-377.

Jenkins DJA, Leeds AR, Slavin B, Mann J, Jepson EM. Dietary fiber and blood lipids: reduction of serum cholesterol in type II hyperlipidemia by guar gum. Am J Clin Nutr 1979;32:16-18.

Jenkins DJA, Reynolds D, Slavin B, Leeds AR, Jenkins AL, Jepson EM. Dietary fiber and blood lipids: treatment of hypercholesterolemia with guar crispbread. Am J Clin Nutr 1980;33:575-581.

Jenkins DJA, Wong GS, Patten R, Bird J, Hall M, Buckley GC, McGuire V, Reichert R, Little JA. Leguminous seeds in the dietary management of hyperlipidemia. Am J Clin Nutr 1983;38:567-573.

Kamath PS, Philips SF, Zinsmeister. Short-chain fatty acids stimulate ileal motility in humans. Gastroent 1989;95:1496-1502.

Kay RM, Truswell AS. Effect of citrus pectin on blood lipids and fecal steroid excretion in man. Am J Clin Nutr 1977;30:171-175.

Kelley MJ, Thomas JN, Story JA. Modification of spectrum of fecal bile acids in rats by dietary fiber. Fed Proc 1981;40:845 abs 3497.

Kennelly JJ, Aherne FX, Sauer WC. Volatile fatty acid production in the hindgut of swine. Can J Anim Sci 1981;61:349-361.

Keys A, Anderson JT, Grande F. Prediction of serum-cholesterol responses of man to changes in fats in the diet. Lancet 1957;2:959-966.

Keys A, Grande F, Anderson JT. Fiber and pectin in the diet and serum cholesterol concentration in man. Proc Soc Exp Biol Med 1961;106:555-558.

Khan AR, Khan GY, Mitchel A, Qadeer MA. Effect of guar gum on blood lipids. Am J Clin Nutr 1981;34:2446-2449.

Kies C. Non-soluble dietary fibre effects on lipid absorption and blood serum patterns. Lipids 1985; 20:802-807.

Kik MJ, vanLeeuwen P, vanDijk JE, Mouwen JM. A small intestinal mucosal biopsy technique in cannulated pigs. J Anim Physiol Anim Nutr 1988;60:123-127.

Kirby RW, Anderson JW, Sieling B, Chen W-J, Kay RM, Miller RE, Rees ED. Oat bran intake selectively lowers serum low-density lipoprotein cholesterol concentrations. Am J Clin Nutr 1981;34;824-829.

Kiriyama A, Okazaki Y, Yoshida A. Hypercholesterolemic effect of polysaccharides and polysaccharide-rich foodstuffs in cholesterol-fed rats. J Nutr 1969;97:382-388.

Kochen MM, Wegscheider K, Abholz HH. Prophylaxis of constipation by wheat bran: a randomized study in hospitalized patients. Digestion 1985;31:220-224.

Koruda M, Rolandelli R, Settle R, Zimmaro D, Hastings J, Rombeau J. The effect of short chain fatty acids (SCFA) on the small bowel mucosa (abs). J Parent Ent Nutr 1987;11(S1):8.

Kritchevsky D, Davidson LM, Shapiro IL, Kim HK, Kitagawa M, Malhotra S, Nair PP, Clarkson TB, Bersohn I, Winter PA. Lipid metabolism and experimental atherosclerosis in baboons: influence of cholesterol-free, semi-synthetic diets. Am J Clin Nutr 1974;27:29-50.

Kritchevsky D, Davidson LM, Krendel DA, Van der Watt JJ, Russell D, Friedland S, Mendelsohn D. Influence of dietary fiber on aortic sudanophilia in vervet monkeys. Ann Nutr Metabol 1981;25:125-136.

Kritchevsky D, Davidson LM, Goodman GT, Tepper SA, Mendelsohn D. Influence of dietary fiber on lipids and aortic composition of vervet monkeys. Lipids 1986;21:338-341.

Kritchevsky D, Davidson LM, Scott DA, VanderWatt JJ, Mendelsohn D. Effects of dietary fibre in vervet monkeys fed "western" diets. Lipids 1988;23:164-168.

Langley NJ, Thye FW. The effect of wheat bran and/or citrus pectin on serum cholesterol and triglycerides in middle aged men. Fed Proc 1977;36:1118 abs 4480.

Leeds AR, Ralphs DNL, Ebied F, Metz G, Dilawari JB. Pectin and the dumping syndrome: Reduction of symptoms and plasma volume changes. Lancet 1981;1:1075-1078.

Lehninger AL. Biochemistry. Second Edition. Worth Publishers, NY, NY. 1975.

Lepage G, Roy CC. Fecal bile acids as a means of assessing the integrity of the enterohepatic circulation of bile acids and their microbial degradation. J Ped Gast Nutr 1988;7:6-9.

Leveille GA, Sauberlich HE. Mechanism of the cholesterol-depressing effect of pectin in the cholesterol-fed rat. J Nutr 1966;88:207-214.

Lowe DM, Tubbs PK. Succinylation and inactivation of 3-hydroxy-3-methylglutaryl-CoA synthase by succinyl-CoA and its possible relevance to the control of ketogenesis. Biochem J 1985;232:37-42.

Mahley RW, Weisgraber KH. An electrophoretic method for the quantitative isolation of human and swine plasma lipoproteins. Biochem 1974;13:1964-1968.

Mahley RW, Weisgraber KH, Innerarity T, Brewer HB, Assmann G. Swine lipoproteins and atherosclerosis. Changes in the plasma lipoproteins and apoproteins induced by cholesterol feeding. Biochem 1975;14:2817-2823.

Marsh JB, Whereat AF. The synthesis of plasma lipoprotein by rat liver. J Biol Chem 1959;234:3196-3200.

Marty JF, Vernay MY, Abravanel GM. Acetate absorption and metabolism in the rabbit hindgut. Gut 1985;26:562-569.

Mattson FH, Grundy SM. Comparison of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. J Lipid Res 1985;26:194-202.

McBurney MI, Thompson LU. In vitro fermentabilities of purified fibre supplements. J Food Sci 1989;54: 347-350.

McBurney MI, Thompson LU, Jenkins DJ. Colonic fermentation of some breads and its implication for energy availability in man. Nutr Res 1987;7:1229-1241.

McLean Ross AH, Eastwood MA, Brydon WG, Anderson JR, Anderson DMW. A study of the effects of dietary gum arabic in humans. Am J Clin Nutr 1983;37:368-375.

McNamara DJ, Kolb R, Parker TS, Batwin H Samuel P, Brown CD, Ahrens EH. Heterogeneity of cholesterol homeostasis in man. J Clin Invest 1987;79:1729-1739.

McNeil NI, Cummings JH, James WP. Short chain fatty acid absorption by the human large intestine. Gut 1978; 19:819-822.

Miettinen TA, Tarpila S. Effect of pectin on serum cholesterol, fecal bile acids, and biliary lipids in normolipidemic and hyperlipidemic individuals. Clin Chim Acta 1977;79:471-477.

Miller TL, Wolin MJ. Fermentation by saccharolytic intestinal bacteria. Am J Clin Nutr 1979;32:164-172.

Mills GL, Taylaur CE. The distribution and composition of serum lipoproteins in eighteen animals. Comp Biochem Physiol 1971;40B:489-501.

National Research Council. Nutrient Requirements of Swine. Ninth Edition, National Academy Press, Washington DC, 1988.

Nestruck AC, Lussier-Cacan S, Bergseth M, Bidallier M, Davignon J, Marcel YL. The effect of protacaval shunt on plasma lipids and lipoproteins in swine. Biochem biophys Acta 1977;188:43-54.

Newman HAI, Zilversmit DB. Quantitative aspects of cholesterol flux in rabbit atheromatous lesions. J Biol Chem 1962;23:2078-2084.

Nyman M, Asp NG. Fermentation of dietary fibre components in the rat intestinal tract. Br J Nutr 1982; 47:357-366.

Palmer GH, Dixon DG. Effect of pectin on serum cholesterol levels. Am J Clin Nutr 1966;18:437-441.

Penagini R, Velio P, Vigorelli R, Bozzani A, Castagnone D, Ranzi T, Bianchi PA. The effect of dietary guar on serum cholesterol, intestinal transit, and fecal output in man. Am J Gastroenterol 1986;81:123-125.

Platt SR, Clydesdale FM. Binding of iron by lignin in the presence of various concentrations of calcium, magnesium, and sinc. J Food Sci 1985;50:1323-1326.

Platt SR, Clydesdale FM. Mineral Binding Characteristics of lignin, guar gum, cellulose, pectin, and neutral detergent fiber under simulated duodenal pH conditions. J Food Sci 52;1987:1414-1419.

Proia AD, McNamara DJ, Edwards DG, Anderson KE. Effects of dietary pectin and cellulose on hepatic and intestinal mixed-function oxidations and hepatic 3-hydroxy-3-methylglutaryl-coenzyme A reductase in the rat. Biochem Pharmacol 1981;30:2553-2558.

Reddy BS, Watanabe K, Sheinfil A. Effect of dietary wheat bran, alfalfa, pectin and carrageenan on plasma cholesterol and fecal bile acid and neutral sterol excretion in rats. J Nutr 1980;110:1247-1254.

Rerat A, Fiszlewicz M, Herpin P, Vaugelade P, Durand M. Measure de l'apparition dans la veine porte des acides gras volatils formes au cours de la digestion chez le porc eveille. C R Acad Sci Paris 1985;300:467-470.

Robinson IM, Allison MJ, Bucklin JA. Characterization of the cecal bacteria of normal pigs. Appl Env Microbiol 1981;41:950-955.

Rodwell VW, Nordstrom JL, Mitschelen JJ. Regulation of HMG-CoA reductase. Adv Lipid Res 1976;14:1-74.

Roediger WE. Utilization of nutrients by isolated epithelial cells of the rat colon. Gastroent 1982; 83:424-429.

Roehrig KL. The physiological effects of dietary fibre - a review. Food Hydrocolloids 1988;2:1-18.

Rombeau JL. Colonic Infusion of short chain fatty acids. J Parent Ent Nutr 1988;12(6):102S-103S.

Roth FX, Kirchgessner M, Muller HL. Energetische verwertung von intracecal infundierter essig-und propionsaure bei sauen - Energetic utilization of intracaecally infused acetic and propionic acid in sows. J Anim Physiol Anim Nutr 1988;59:211-217.

Roy CC, Weber AM. Clinical implications of bile acids in paediatrics. Clin Gastroent 1977;6:377-395.

Ruppin H, Bar-Meir S, Soergel KH, Wood CM, Schmitt MG. Absorption of short chain fatty acids by the colon. Gastroent 1980;78:1500-1507.

Sakata T. Stimulatory effect of short chain fatty acids on epithelial cell proliferation in the rat intestine: a possible explanation for the trophic effects of fermentable fibre, gut microbes and luminal trophic effects. Br J Nutr 1987;58:95-103.

Sambrook IE. Studies in digestion and absorption in the intestines of growing pigs. Measurements of the flow of total lipid, acid-detergent fibre and volatile fatty acids. Br J Nutr 1979;42:279-287.

Sauer WC, Jorgensen H, Berzins R. A modified nylon bag technique for determining apparent digestibilities of protein in feedstuffs for pigs. Can J Anim Sci 1983; 63:233-237.

Sauer WC. Factors influencing amino acid availability for cereal grains and their components for growing monogastric animals. PhD Thesis, University of Manitoba, Winnipeg, Manitoba, 1976.

Scrutton MC, Utter MF. The regulation of glycolysis and gluconeogenesis in animal tissues. Ann Rev Biochem 1968;37:249-302.

Sheltawy MJ, Losowsky MS. Determination of fecal bile acids by an enzymatic method. Clin Chim Acta 1975; 64:127-132.

Shuttler SM. Investigations into the hypocholesterolaemic effect of legumes. PhD Thesis, University of Reading, England, 1989.

Simons LA, Gayst S, Balasubramaniam S, Ruys J. Long-term treatment of hypercholesterolaemia with a new palatable formulation of guar gum. Atherosclerosis 1982; 45:101-108.

Slack J. Risks of ischaemic heart-disease in familial hyperlipoproteinaemic states. Lancet 1969; 2:1380-1382.

Smith RG, Rowe MJ, Smith AN, Eastwood MA, Drummond E, Brydon WG. A study of bulking agents in elderly patients. Age Ageing 1980;9:267-271.

Sperber I, Bjornhag G, Ridderstale Y. Function of proximal colon in lemming and rat. Swedish J Agric Res 1983; 13:246-256.

Stephen AM, Cummings JH. The microbial contribution to human fecal mass. J Med Microbiol 1980;13:45-56.

Stevens CE. Comparative physiology of the digestive system. In: Dukes Physiology of Domestic Animals, 9th Edition. Swenson MJ Ed. Cornell University Press, Ithica, NY. 1977:216-230.

Storch K, Anderson JW, Young VR. Oat bran muffins lower serum cholesterol of healthy young people. Clin Res 1984;34:740(A).

Story JA. Dietary fibre and lipid metabolism. Proc Soc Exptl Biol Med 1985;180:447-452.

Swain JF, Rouse IL, Curley CB, Sacks FM. Comparison of the effects of oat bran and low-fiber wheat on serum lipoprotein levels and blood pressure. N Eng J Med 1990;322:147-152.

Thacker PA, Bowland JP. Effects of dietary propionic acid on serum lipids and lipoproteins of pigs fed diets supplemented with soybean meal or canola meal. Can J Anim Sci 1981;61:439-448.

Thacker PA, Salomons MO, Aherne FX, Milligan LP, Bowland JP. Influence of propionic acid on the cholesterol metabolism of pigs fed hypercholesterolemic diets. Can J Anim Sci 1981;61:969-975.

Tiedink GM, Katan MB. Frozen storage of serum does not affect cholesterol and triglyceride concentration in lipoproteins as separated by gradient ultracentrifugation. Clin Chem 1988;34:593-594.

Tomlin J, Taylor JS, Read NW. The effects of mixed faecal bacteria on a selection of viscous polysaccharides in vitro. Nutr Rep Int 1989;39:121-135.

Trowell H, Southgate DA, Wolever TM, Leeds AR, Gassul MA, Jenkins DJ. Dietary fibre redefined. Lancet 1976; 1:967.

Tsai AC, Elias J, Kelley JJ, Lin RSC, Robson JRK. Influence of certain dietary fibers on serum and tissue cholesterol levels in rats. J Nutr 1976;106:118-123.

Tuomilehto J, Karttunen P, Vinni S, Kostiainen E, Uusitupa M. A double-blind evaluation of guar gum in patients with dyslipidaemia. Hum Nutr Clin Nutr 1983; 373:109-115.

Tuomilehto J, Silvasti M, Aro A, Koistinen A, Karttunen P, Gref CG, Ehnholm C, Uusitupa M. Long term treatment of severe hypercholesterolaemia with guar gum. Atherosclerosis 1988;72:157-162.

Turner PR, Tuomilehto J, Happonen P, La Ville AE, Shaikh M, Lewis B. Metabolic studies on the hypolipidaemic effect of guar gum. Atherosclerosis 1990,81:145-150.

Umesaki Y, Yajima T, Yokokura T, Mutai M. Effect of organic acid absorption on bicarbonate transport in rat colon. PFlugers Arch 1979;379:43-47.

Vahouny GV, Tombes R, Cassidy MM, Kritchevsky D, Gallo LL. Dietary fibers V. Binding of bile salts, phospholipids and cholesterol from mixed micelles by bile acid sequestrants and dietary fibers. Lipids 1980; 15:1012-1018.

Vahouny GV, Khalafi R, Satchithanandam S, Watkins DW, Story JA, Cassidy MM, Kritchevsky D. Dietary fiber supplementation and fecal bile acids, neutral steroids, and divalent cations in rats. J Nutr 1987; 117:2009-2015.

Vahouny GV, Satchithanandam S, Chen I, Tepper SA, Kritchevsky D, Lightfood FG, Cassidy MM. Dietary fibre and intestinal adaptation: effect on lipid absorption and lymphatic transport in the rat. Am J Clin Nutr 1988;47:201-206.

VanLeeuwen P, Sauer WC, Huisman J, vanKleef D, denHartog LA. Ileo-caecal re-entrant cannulation in baby pigs. J Anim Physiol Anim Nutr 1988;59:56-63.

Van Soest PJ, Jeraci J, Fosse T, Wrick K, Ehle F. Comparative fermentation of fibre in man and other animals. In: Fibre in Human and Animal Nutrition. G Wallace, L Bell eds. Bulletin 20. The Royal Society of New Zealand, Wellington. 1982:75-80.

Varel VH, Fryda SJ, Robinson IM. Cellulolytic bacteria from pig large intestine. Appl Environ Microbiol 1984; 47:219-221.

Vega GL, Groszek E, Wolf R, Grundy SM. Influence of polyunsaturated fats on composition of plasma lipoproteins and apolipoproteins. J Lipid Res 1982;23:811-822.

Venter CS, Vorster HH, Cummings JH. Effects of dietary propionate on carbohydrate and lipid metabolism in healthy volunteers. Am J Gastroent 1990;85:549-553.

Vigne JL, Lairon D, Borel P, Portugal H, Pauli AM, Hauton JC, Lafont H. Effect of pectin , wheat bran and cellulose on serum lipids and lipoproteins in rats fed on a low- or high-fat diet. Br J Nutr 1987;58:405-413.

Wells AF, Ershoff BH. Comparative effects of pectin N.F. administration on the cholestero-fed rabbit, guinea pig, hamster and rat. Proc Soc Exp Biol Med 1962;111:147-149.

Whitney EN, Hamilton EMN. Understanding Nutrition. Fourth Edition. West Publishing, St. Paul MO, 1987.

Wilkenson CF, Hand EA, Fliegelman MT. Essential familial hypercholesterolemia. Ann Int Med 1948;29:671-686.

Wood JD, Migicovsky BB. Fatty acid inhibition of cholesterol synthesis. Can J Biochem Physiol 1956;34:861-865.